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ANNOUNCEMENT

The Bryn Mawr College Monographs will be issued in two series; the first, the ***Monograph Series,*** containing articles that appear here for the first time; the second, the ***Reprint Series,*** containing reprints of articles that have appeared in other journals.

It is proposed to publish these monographs in separate numbers at irregular intervals as material is accumulated. The numbers will be combined into volumes of about 500 pages.

The monographs are edited by a committee of the Faculty of Bryn Mawr College, consisting at present of President M. Carey Thomas, ***ex-officio,*** Professor Thomas Hunt Morgan (chairman), Professor Lindley Miller Keasbey, and Professor David Irons.

MAY 24 1904

BRYN MAWR COLLEGE MONOGRAPHS

REPRINT SERIES, Vol. I., No. 3

CONTRIBUTIONS FROM THE BIOLOGICAL LABORATORY

BRYN MAWR, PENNA., U. S. A.
April 1904

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The Relation Between Normal and Abnormal Development of the Embryo of the Frog, as Determined by the Effect of Lithium Chloride in Solution.

By

T. H. Morgan.

With Plates XXIII and XXIV.

Eingegangen am 15. Mai 1902.

The marked effect that lithium salts may have on the developing organism was first shown by HERBST in his study of the action of this metal on the sea-urchin's egg. A typical embryo was formed that he has called the lithium larva. GURWITSCH, BATAILLON, and Mm. RONDEAU-LUZEAU have more recently studied the effect of lithium salts on the development of the frog's egg, and have shown that it has a distinct effect on the development but whether its action produces a distinct type of embryo is not altogether clear from their results, although GURWITSCH seemed inclined to think that his radial type of embryo was the result of the peculiar action of the lithium salt that he used. Whether there is in reality a radial type produced in this way is a question that will be discussed later.

In the series of experiments that I carried out in the spring of 1902, I used only lithium chloride but in the experiments carried out during the present spring, I have employed not only this salt, but also a number of other lithium salts, as well as other solutions for comparion.

The eggs were in all cases collected from a pond in the vicinity, where they had been laid and fertilized normally. Some of the eggs were put into the solutions while in the two- and four-cell stages, others in the later segmentation stages. Ordinary spring water was used to which was added in the earlier experiments 0.4, 0.5, 0.6, 0.7, 0.8 percentages of lithium chloride. The eggs were kept in these solutions until they were removed directly to be killed in a three percent formalin solution. Later they were passed through alcohols, imbedded, cut, stained in borax carmine followed by LYON's blue.

It may be stated that one of the most obvious effects of the salt is to delay the development. Eggs in the two- and four-cell stages are much more affected by the solutions than are eggs in the later segmentation stages, and those that are beginning to gastrulate are still less effected. Solutions that are strong enough to prevent the formation of the embryo when two-cell stages are employed do not prevent the embryo from appearing when later segmentation stages are put into the same solution. In general, the effect of the salt is to prevent the downward movement of protoplasm from the upper hemisphere of the egg, and this brings in its train a difficulty in carrying out the gastrulation process, so that embryos in the stronger solutions do not pass beyond the stages shown in Fig. *R* especially when two-cell stages are immersed. When older stages are used embryos like those in Figs. *M*, *T*, *W*, are commonly produced. While embryos of these two kinds form by far the majority of cases they are not as interesting as those of another more modified type, shown in Figs. *A*—*L*; and these rarer forms will, therefore, be first described. Embryos of this last type were sometimes obtained when late segmentation stages were immersed in lithium chloride solutions. These embryos, as shown in Figs. *A* and *L* have a small black area at the top of the egg which is surrounded by a deep groove. The region of the egg below this is of a bluish-yellow, or slate colored tint. On one side this band is much broader than on the other, and at the lower edge of the broadest part there is a crescentic shaped depression. This is the opening into the archenteron. The lower part of the egg is made up of the yellowish yolk-cells.

By means of sections it is not difficult to interpret these embryos. The entire upper part of the egg, including all the black cells of the upper hemisphere, has sunk into the interior of the egg. There only remains the small black region at the top of the egg. As these cells have been drawn into the interior, the superficial yolk cells at the sides have been pulled upwards over the outside of the upper hemisphere of the egg. These cells make up the slate-colored. middle zone of the egg. The blastopore that appeared (whether at a stage before or after the inturning began I can not state), continues into the archenteron that extends deep in the interior of the yolk. A series of three cross-sections of the egg shown in Fig. *A* are represented in Figs. I, II, III. These cross-sections are nearly in a plane at right angles to the egg as it stands on the paper, and cut across the line marking the notochord. The plane of the sections tilts a little upwards, towards the far side of the egg. The first of these sections, Fig. I, passes through the slate-colored region. This part forms a crescent enclosing the central darker area. Beginning with the crescent part, we find on the outer surface (the upper side of the figure) a single layer of narrow cells filled with yolk. This layer is made up of the yolk-cells that I have described as having been drawn up, when the top of the egg sank inwards. It marks the slate-colored zone of the surface view. About the middle of this layer, and just below it, is the cross-section of the notochord. On each side of the notochord is a layer of mesoderm extending out into the wings of the crescent. On the inner side of this crescentic part there is a broad medullary plate in process of rolling in.

The other piece, the lower part of the section, nearly enclosed by the crescent, contains the other side of the medullary plate. The two plates are continuous at a deeper level in the embryo. In the middle of this piece, the innermost end of the archenteron is seen. The loose cells, lying between the two parts of this section, are ectoderm cells that have been crowded out from the surface.

The next section, Fig. II, is nearer the middle of the slate-colored band. The two pieces have become one, since just below this level the ectoderm of one piece continues into that of the other. The ectoderm forms the dark ring in the interior of the egg. The two medullary plates have also united into a single tube. It is here that the medullary plate is to be thought of as turning back upon itself. The notochord, the mesoderm, and the archenteron are found in approximately the same positions as in the last section.

The next figure, Fig. III, is still further down, in fact at the very bottom of the medullary tube, where it turns back upon itself as explained above. We now see the notochord cut twice, once on the outer side, and once on the inner side of the medullary tube. Mesoderm, with some ectoderm on one side, fills the interior of the section. On the sides, the yolk-cells of the lower hemisphere begin to appear. Sections further down (which are not here figured) show that the medullary groove comes to an end. The notochord on the inner side of the inner medullary groove becomes continuous with that on the outer surface. The archenteron also extends outwards to open at the small blastopore on the surface of the egg. Below this the solid yolk-cells of the lower hemisphere make up the whole of the sections.

A longitudinal section through an embryo very similar to the last is shown in Fig. *V*. This section shows the archenteron opening by the blastopore on the left. On the right side there is an opening, higher up, into the top of the egg. This upper cavity is lined by a thick ectoderm, which forms in part the broad medullary plate that bends around the inner end of the cavity. The upper and left upper sides of this section are through the slate colored band, and, had the section been exactly in the middle line, the notochord would lie immediately below the layer of slate-colored cells that cover this part. Some loose ectoderm cells partly fill up the interior of the ectodermal cavity, and come to the surface of the egg at its opening. It will be seen in this section that all the ectoderm is in the interior of the egg, and much of the endoderm on the outside.

The embryo shown in Fig. *B* was also cut into cross-sections. This embryo differs from the last mainly to the smaller extent to which ectoderm has been turned in at the top of the egg. The slate-colored band is well defined, and a depression along its middle part shows where the notochord runs beneath its surface. Only one of the the cross-sections of this embryo is drawn, Fig. IV. It is, in many ways, similar to Fig. I. The relation of the section to the egg can be best understood by reference to the side view of a similar egg shown in Fig. *L*. This figure shows to advantage the relation between the black cap of the egg and the slate-colored zone. If a cross-section were made through the middle of this egg, at about the level of the middle of the slate-colored zone, it would resemble that shown in Fig. IV. The crescentic shaped piece forming the upper part of the section is through the slate-colored area (the left side of Fig. *L*). It appears as though partly wrapped around the black area. In fact that part of the black area that comes to the surface is the part not surrounded by the arms of the crescent. We get the impression that the slate-colored area is rolling up over the black part of the egg, as the latter sinks into the interior; the inrolling appearing to take place faster on the side where the embryo is formed, than elsewhere.

The section is almost exactly like that drawn in Fig. I, except that the rounded part is larger and the notochord is present in both pieces. The section also shows the medullary plate, cut twice. On each side of the plate, the neural crest is seen.

The egg drawn in Fig. *C* has turned in almost all of its black field, and the slate-colored region bulges far outwards. Cross-sections show that it essentially like the eggs shown in Figs. *A* and *B*.

Figure *D* is interesting, because only a part of the medullary groove is rolled into the interior of the egg; the anterior part is seen on the exposed black surface. Cross-sections through the anterior part show an almost normal embryo (the ectoderm is somewhat loose in places). Passing into the region of the slate-colored band the cross-sections show that here the posterior part of the medullary plate is covered over by the upturned edge of the band.

Figure *E* shows the side view of an embryo with a very large opening (on the left in the figure) into the archenteron. The slate-colored region is scarcely developed, and the black region is hardly at all turned in above the blastopore. A longitudinal section of this embryo is shown in Fig. VI. The large opening into the archenteron (to the right in this figure) is conspicuous. The part just above shows a slight tendency to turn in. The thick ectoderm on the top of the section is the medullary plate. There is a slight inturning at the anterior end of the medullary plate; the anterior end of the black area.

The next figure, Fig. *F*, shows an embryo in which practically all of the black hemisphere of the egg has heen turned into the interior. Only a small region of loose cells (on the opposite side of the egg from that shown in the figure) indicates where the inturning has occurred. The medullary plate has been formed inside, somewhat as in Fig. V. The archenteron is short and the blastopore is represented by a small triangular hole seen in the figure.

The next figure, Fig. *G*, is much like the last, but the blastopore is wider.

The next figure, Fig. *H*, is much like Fig. *B*. A longitudinal section, through the median plane of this embryo, is shown in Fig. VII. It will be seen in this section that only a small part of the black hemisphere has been covered over by the slate-colored area. This section also shows how far the archenteron may extend up into the upper hemisphere.

Figures *I* and *J* represent posterior and side views of the same egg. In the latter it will be noticed how much the slate-colored area protrudes, which is due here, as in all other cases, to the part that is turned into the interior of the egg bulging out the covering cells in that region. Cross-section of this egg show that it is almost exactly like the egg drawn in Fig. *A*. The embryo figured in Fig. *K* and *L* is also like the last.

An embryo, which externally was very similar to Fig. *K*, was cut into longitudinal sections, one of which is shown in Fig. VIII. Here the whole top of the egg has sunken into the interior, but it is difficult to tell from sections in this plane how far the medullary plate has developed. A crowd of loose cells fills the opening of the inturned, ectodermal cup. The archenteric invagination has not progressed very far, or if it had, it has been pulled out again. There was also another egg of this same lot in which the ectoderm had turned in as in the last case, but although there was a depression on the surface, where the blastopore should form, none was present, as sections showed. Here also it is possible that the drawing upward of the endoderm cells has either prevented the formation of the archenteron, or if it began to develop, it has been pulled out again. The set from which the last three eggs were taken differed from the preceeding ones in that they had been put into the four percent solution at an earlier stage of segmentation. The general result is the same, but in the less complete development of the nervous system, and in the less development of the archenteron, they stand intermediate between the preceeding embryos and a very common type drawn in Figs. *Q* and *R*, which will now be described.

Many of the eggs were put into the solutions at the two- and four-cell stages. Some of these underwent a series of changes that are in many respects similar to those just described, but they did not develop so far. An embryo of this sort is shown in Fig. *Q*. There is a mass of black cells at the top, surrounded by a groove, which separates this mass from a ring, showing the slate-blue color. This forms a band around the egg just above the equator. In this egg the band is sharply limited below by a groove which seems to correspond to an archenteric invagination. The groove does not extend quite around the egg. Vertical sections show that the black cells at the top are sinking in to form a sort of column (see Fig. X) bordered by the upper groove seen in the surface view. There is no evidence of the formation of the medullary plate in the interior.

In Fig. *R*, a side view of another egg, similar in many ways to the last, is shown. The next figure is also similar to these, but there is no archenteric invagination below the slate-colored band. Sections of this egg are similar to the section shown in Fig. X. In this the ectoderm has turned into the interior, and its central part shows evidence of hollowing out, which is completely accomplished in some other eggs of this kind. In this section, Fig. X, it will be noticed that the yolk-cells are drawn up at the sides to the top of the egg, and also that those immediately around the central ectodermal mass are flattened around the surface of the interior, ectodermal ball.

It is not uncommon to find eggs in these solutions in which there is a sharp line between the black hemisphere and the yolk-cells, and in some of these an embryo appears in the black part, as shown in Fig. *M.* A broad medullary plate extends to the top, or nearly to the top, of the black cap. Sections of this embryo show that the ectoderm is flattened to form the medullary plate which extends far out at the sides. It is thinner in the middle. Beneath this lies a thick mesodermal sheet, continuous at the sides with the mesoderm differentiating out of the yolk-cells. The notochord can not be identified with certainty. A wide archenteron is present under the embryo in the upper part of the yolk, and it opens behind under the projecting part of the embryo. Around the sides the inturned edge also represents a blastopore-rim.

Another somewhat similar embryo is represented in Fig. *N.* In this, a long and narrow groove is seen extending to the top of the black portion. Cross-sections show on each side of this groove a well developed medullary plate. Beneath it, in the middle line, there is a distinct notochord, which in places is closely united with the dorsal wall of the archenteron. Mesoderm lies at each side. The yolk around the sides of the black-white rim is dead and vacuolated, although not so much so at the posterior end. The black-white edge itself forms a thickened ridge. A well developed archenteron lies above the yolk under the embryo.

In only a few cases did eggs, that had been put into the salt solutions in the two-celled stage, produce an embryo on the black portion. One embryo, in which this occured, is shown in Fig. *O.* The egg had been put into a 0.5 per cent solution of lithium chloride at the two-cell stage, and after six days had only developed as far as seen in the figure. Sections show that the embryo lies quite excentrically on the black portion, and that one side is developed better than the other. This embryo is abnormal in several respects. The medullary plate is poorly developed, the notochord is disproportionately large, and the mesoderm on each side is irregularly developed. Along one edge (the right in the figure) the archenteron comes to the surface just beyond one side of the medullary plate. The opposite black-white edge (to the left and below in the figure), shows the ectoderm wall becoming thin, and not turning in, although there are a few yolk-mesodermal cells just beneath the edge. The archenteron, that lies beneath the embryo, is extremely irregular.

Another egg of this same series is shown in Fig. *P*. The medullary plate is better developed; the notochord is large and lies somewhat excentrically, and cells of the dorsal wall of the archenteron extend, in places, up into the notochord. The archenteron itself is well developed. There is no sharp line at the boundary between the black and the white cells, and the ectoderm cells in this region are often spherical and are loosely held together.

Somewhat different types of embryos are represented in the following figures. These types are quite common amongst the embryos in the solutions. The embryo shown in Fig. *T* has a black hemisphere separated on one side (that shown in the figure), by a sharp line from the yolk. A vertical section of this egg is shown in Fig. IX. The upper third of the egg is a solid mass of small cells. A short archenteric invagination is seen at one side. There are no indications of the formation of a medullary plate, or of other parts of an embryo.

The next embryo, Fig. *U*, is about four-fifths black with the yolk exposed only over the remaining fifth. A sharp border separates the black from the white. At one place the black projects backwards over the white. A vertical section is shown in Fig. XI. The upper side, to judge from the greater thickness of the ectoderm, corresponds to the dorsal side of the embryo, but there are no indications of the formation of a medullary plate. The opposite side of the embryo has grown into the yolk, nearly cutting off the yolk-plug from its connection with the rest of the yolk. Ingrowths of this sort are frequent in eggs of this kind.

The next figure, Fig. *V*, shows a solid black cap prolonged on one side. A section in the plane of the paper is shown in Fig. XII. A deep archenteron extends under one side of the egg. Above it there is a layer of mesoderm, and beyond this, on the surface, is the thickened ectoderm which does not show any indications of forming a medullary plate. The peculiar crack in the yolk-cells, leading from the archenteron, should be noticed, since it is a common occurence in eggs of this kind. It may represent an extension of the archenteron into the yolk, or it may represent the primoidium of the liver.

In Fig. *W* two tail like extensions have grown back from the black hemisphere over the surface of the yolk. Cross-sections of this embryo show that the ectoderm is thickened on one side (that turned towards the observer in the figure); beneath it lies an archenteron, but there are no further signs of the formation of an embryo.

The next figure, Fig. *X*, is like the last, but has a single growth backwards. Section of this embryo are very much like that shown in Fig. XII.

The last three eggs are from a set that had been put at the two-cell stage into a 0.5 per cent solution of lithium chloride. The backgrowth, shown in Figs. *U*, *W*, *X*, appears to be a continuation from the dorsal lip of the blastopore. The projection has ectoderm above, endoderm below, and mesoderm between these two.

The preceeding description of a few of the typical kinds of embryos that developed in the salt-solutions will give a general idea of the effects that the salt produces. The most unique embryos are those first described, in which there is a complete inversion of the layers. The other types approach those that GURWITSCH has obtained, more especially those drawn in Figs. *T* to *X*. In nearly all these series, and especially those in the diluter solutions, and when later stages are placed in the solutions, many normal embryos develop, in addition to the abnormal kinds here represented. But when the solutions are so strong that embryos of the first type appear it is rare to get also normal embryos. The following records will show what the general effect of the different solutions, used in the spring of 1902, produced.

The Different Kinds of Embryos Produced in the Solutions.

First Set.

Eggs in a late segmentation stage were put into a 0.4 per cent solution of lithium chloride, IV. 12, 1902. They were preserved at the four following periods.

IV. 15. In most eggs at this time the blastopore is present at or below the equator of the egg. Some few eggs show the anterior end of the medullary plate as in Fig. *M*. In some cases the embryos lie a little obliquely on the black part of the egg.

IV. 18. There were a number of embryos like those in Figs. *A*, *B*, *H*, *I*. Others were like those in Figs. *X* and *U*, and others still had a black hemisphere on which lay a short medullary plate that extended to the top of the egg.

IV. 19. The main types of embryos found at this time are: a) some nearly normal with a large blastopore and with the medullary plate turning in; b) some with still larger blastopore and no medullary plate visible; c) others like Figs. *A*, *F*, *G* (and these were found in some of the same clusters in which a) and b) are present).

IV. 20. In most of these embryos the blastopore was closing or closed, but only rarely is there an indication of the medullary plate on these eggs. A few eggs like those in Figs. *A*, *F*, *G* were present.

A number of other eggs that had been put into 0.4 lithium chloride in the late segmentation stages had covered over the white hemisphere; in some the blastopore has almost completely or even completely closed. In those that showed the medullary plate, it was found, when the eggs were cross-sectioned, that the embryos were abnormal to various extents. Many of these eggs showed no evidence of forming a medullary plate or definite embryo.

Second Set.

Eggs in the 2- and 4-cell stages were put into a 0.4 per cent solution of lithium chloride, IV. 12, 1902. The preserved eggs showed the following results.

IV. 16. There were two main kinds of embryos at this time: a) the black hemisphere occupies the top of the egg, as in Fig. *T*, and is often sharply marked off from the yolk; b) most eggs have a smaller black cap separated by a line from a lighter slate-colored region, as in Fig. *S*.

IV. 18. Here also there were two principal kinds of embryos: a) like those shown in Figs. *T*, *V*, *W*, *X*; b) others with a black cap and a slate-colored band as in Fig. *R*.

IV. 19. Most of the eggs preserved have a black cap sinking in, like those shown in Figs. *R* and *S*. This black cap is less compact on the surface than in earlier stages.

IV. 20. The eggs have not developed any further, and appear as before.

Third Set.

Eggs in the four-cell stage were put into a 0.4 per cent solution of lithium chloride. IV. 12, 1902.

IV. 20. There are two kinds of embryos; most have a black upper hemisphere like that in Fig. *T*; others are further closed in, as in Figs. *U* and *X*; b) a few show the beginning of the slate-colored band, which is not yet sharply limited above and below.

IV. 21. Nearly all the eggs are like those in Figs. *U*, *W*, *X*. A few have the blastopore more closed. Very rarely an egg, like that in Figs. *V* and *X*, shows faint indications of a short medullary plate on the black hemisphere. A very few eggs have the slate-colored band.

IV. 23. Most of the eggs are in the same condition as before, but more of the eggs show the black cap sinking into the interior.

IV. 24. The embryos have not developed any further. The black cap is becoming furrowed, and is losing 'is deep black color, due most probably to the cells becoming looser.

IV. 25 and 26. No further development has taken place.

Fourth Set.

Eggs in the two-cell stage were put into a 0.5 per cent solution of lithium chloride; IV. 12, 1902.

IV. 17. The eggs were at this time in good condition, but did not yet show any indication of the medullary plate. The eggs were like those in Figs. *U*, *W*, *X* (rarely one like Fig. *R*).

IV. 18. One lot was particularly good, in which the embryos were like those in Figs. *U*, *W*, *X*. In one, like Fig. *U*, faint traces of a medullary fold appeared on the projecting part.

IV. 20. Most of the eggs are now not in good condition. One only showed a medullary fold on the black hemisphere.

IV. 21. The solution had been diluted on the 19. The bunches had behaved differently: a) in one bunch the eggs had a black hemisphere, like Fig. *T*; b) in another the black cap is sinking in; c) in another the eggs were like Fig. *B*. Two small bunches showed the blastopore not closed, and the anterior end of the embryo extending forward on the black part as in Fig. *P*.

IV. 25. These also has been put in a diluted solution on the 19th but were in poor condition, and had not developed further.

Fifth Set.

Eggs in a late segmentation stage were put into a 0.6 per cent solution of lithium chloride, IV. 12, 1902.

IV. 17. These eggs showed excellently the inturning of the black cap; nearly all the eggs being like those in Figs. *F*, *G*.

Sixth Set.

Eggs in the two-cell stage were put into a 0.6 per cent solution of lithium chloride, IV. 12, 1902.

IV. 21. The development of these eggs had been arrested in the segmentation stages. Many whitish cells are present in the upper hemisphere, each with a white border.

Seventh Set.

Eggs, in a late segmentation stage, were put into a 0.7 per cent solution of lithium chloride. IV. 13, 1902.

IV. 16. No change was observable, although the eggs may have segmented further.

IV. 21. The development had stopped, and the black cells were losing color.

Eigth Set.

Eggs in a late segmentation stage were put into a 0.8 per cent solution of lithium chloride, IV. 13, 1902.

IV. 17. The dorsal lip of the blastopore appeared in a few eggs but further development seems to have stopped.

Ninth Set.

Eggs in the 4-cell stage were put into an 0.8 solution of lithium chloride, IV. 13, 1902.

IV. 21. The development had stopped at an early segmentation stage. The next day the segmentation appeared to have gone a little further, but on the next day the eggs appeared to be dead.

There were three other series of eggs comparable in every way to those in the preceeding list, but since they showed nothing new it is not necessary to give an account of them.

Conclusions.

One of the most characteristic effects of the solutions of lithium chloride is to cause the cells of the upper hemisphere to turn into the interior of the egg. If the eggs are in a late segmentation stage when put into the solution a medullary plate may develop on the inturned part, and a notochord and mesoderm appear just beneath the surface of the slate-colored area, Figs. *A* to *L*. If the eggs are in the two- or four-cell stage when put into the solution, the medullary plate does not appear, Figs. *Q*, *R*, *S*. An archenteric invagination may appear on one side of the egg just below the slate-colored area.

Many of the eggs in the solutions do not turn in the upper part of the egg-hemisphere, but a sharp line is formed between the black and the white cells at or often above the equator of the egg, Fig. *T*. This line of separation corresponds to an inturned edge of a circular blastopore, whose position at or above the equator of the egg is explained by the lack of downgrowth of the material of the upper hemisphere. Some of these eggs produce, on the upper hemisphere, the anterior part of the medullary folds which sometimes extend quite to, or even beyond the top of the egg. It would be erroneous to conclude from the position of this medullary fold that the normal medullary fold also extends to the top of the egg.

Review of Literature.

In 1895 I tried the effect of a number of salt-solutions — barium, potassium, calcium, and sodium chlorides — on the development of the frog's egg, and obtained embryos showing all conditions of spina bifida. The eggs were put into the solutions at the time when the blastopore had just appeared. In the most extreme cases the blastopore extended around the equator of the egg, and »ring-embryos« were produced.

In the following year, 1896, HERTWIG carried out a more extensive series of experiments and also obtained embryos showing spina bifida, and others showing abnormalities in the medullary plate. He put the eggs into the solutions one hour after fertilization.

GURWITSCH, in 1895/6, tried the effect if a number of different solutions, more especially of halogen salts on the developing frog's egg. He found that sodium and lithium were most effective. The potassium salts did not act well. The main effect of the chloride of lithium was to cause abnormalities in the position and development of the blastopore and of the brain, but especially of the former.

GURWITSCH found that eggs of Rana fusca in 0.8 and 0.7 per cent solutions of lithium chloride showed a retardation of the segmentation of the cells in the white hemisphere. In many cases the egg remained quite unsegmented; in other cases only the first furrow extended into it. His most satisfactory results were with a 0.5 per cent solution. The lips of the blastopore appeared around the sides of the egg and in this respect it differed from the normal egg, in which, as the lips of the blastopore extend outwards, the opening of the blastopore becomes reduced. Another peculiarity that he observed was that the lateral lips of the blastopore do not always correspond with the line between the black and the white cells, but lie somewhat higher on the egg, as shown by his Fig. 3[1]). The egg becomes marked in consequence into an upper and a lower hemisphere. Vertical sections of an egg of this sort show a small segmentation cavity in the upper hemisphere with a thick roof. The cells forming the sides of the floor of the segmentation cavity appear to be drawing into the interior to fill up the cavity. These cells are wedge shaped with their apices turned towards the the equatorial groove. GURWITSCH claims that the embryos are at this time radially symmetrical, but I believe on insufficient evidence, for although the bilateral form may be less marked then in the normal egg, yet my results appear to indicate that such a bilateral condition is present. Later in some of these eggs the equatorial ring which marks the edge of the blastopore begins to extend over the surface of the lower hemisphere, and a split appears in the yolk beneath the down-growing edge. This split GURWITSCH believes is caused by the drawing apart of the yolk cells. The medullary folds may next appear on the dark hemisphere, and are often quite excentric in position — in extreme cases even lying along one side of the equatorial band. A large yolk-mass continues to project at the lower part, but there is no evidence of the formation of an embryo showing spina bifida, as HERTWIG and I had found for eggs in other salt-solutions. The one apparently exceptional case that GURWITSCH describes (Figs. 11 and 35) in which besides an axial embryo a thickening of ectoderm is formed around the edges is not, as he supposes, a spina bifida form but is, as my results show clearly, due to a folding upward at the posterior end just above the blastopore invagination. GURWITSCH compares his results with the normal development. His attempt to find a parallel between what he supposes to be a radial type of abnormal development of the frog with what he calls the radial type of gastrula of amphioxus does not appear to me to be particularly instructive, since the gastrula of the amphioxus is not radial, and neither do I think are these lithium embryos. In general, comparisons of this sort can not have much value, since the forms are too remote from each other in their method of development to allow of comparisons of any worth.

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[1]) This figure resembles my Fig. *R*, in which the upper black line is not the blastopore rim at all, as GURWITSCH appears to think, but the edge of the inturned black cap.

Gurwitsch calls attention to the lack of down-growth from the upper hemisphere shown by these embryos, but fails, to realize the importance that this fact may have on the subsequent location of the embryo. This lack of down-growth accounts for the high position assumed by these embryos on the black hemisphere, and also, in some degree, for the absence of the spina bifida condition in the embryos in these lithium solutions. The halves of the body of the spina bifida embryos are formed out of material that has been pushed down to the equator of the egg. Since this material lies in the lithium eggs at the top of the egg the opportunity to produce the lateral halves is not present. This statement refers more particularly to eggs like those in Fig. *T*. Moreover in the spina bifida embryos the head is laid down at or just above the equator of the egg because the material out of which the head is to develop has been carried down to this level. On the other hand in these lithium embryos the head, as has been stated, often reaches to the very top of the black hemisphere. There is in consequence an entirely different distribution of formative material.

The unsymmetrical position assumed quite often by embryos of the type of Fig. *N* is difficult to explain. We can only ascribe this, hypothetically, to an unequal distribution in the amount of material, or to the greater injury of the material on one side.

Gurwitsch's criticism of the concrescence theory as applied to the normal embryo from the point of view of his results on these lithium embryos carries little weight with it, since the early stages in these embryos are so different from the normal that any conclusion from them as to what takes place in the normal is likely to go wide of the mark. The same criticism may be made in regard to his conclusion of the position of the normal embryo on the egg, and the imagined absence of overgrowth of the dorsal lip. I have insisted on several occasions that a mistake of this same sort has been made in drawing conclusion from the spina bifida embryos, namely as to the extent of the concrescence of the normal embryo, as maintained by Hertwig and by Roux.

The effect of salt-solutions on development was also tried by C. B. Wilson who placed frog's eggs, in different stages, in solutions of 0.6 per cent sodium chloride, and of 0.3 per cent potassium chloride. He found that the yolk-cells are more easily injured than are those of the upper hemisphere. He states that the eggs of Chorophilus, which develop quite quickly, are less injured at first by the solution, but the after-effect is worse. In this species the injury produced by the salt is greater, the later the stage of development the eggs are in when immersed. The power to resist these solutions can be increased by beginning with a weak solution, 0.5 per cent sodium chloride, and gradually increasing it to 0.6 and to 0.9 per cent. An embryo can thus be acclimatized to live in a solution that would kill it were it placed directly in the stronger solution.

Bataillon induced the segmentation of the unfertilized eggs of Rana esculenta by placing them in a serum, that was isotonic with solutions of sugar and of salts that produce the same effect. When fertilized eggs were put into solutions of sodium and potassium chloride and sugar solutions of different percentages, embryos were obtained similar to those described by Morgan, Hertwig, and Gurwitsch. The blastopore appeared around the equator of the egg, and later the anterior part of the medullary folds appeared on the black region and extended around the large blastopore producing a condition of spina bifida at the posterior end.

Bataillon found that in solutions of sodium bromide and sodium chloride the development progressed normally at a temperature of 15 degrees, but at a higher temperature (20°), equatorial gastrulation took place, and the older embryos from such eggs had an exposure of yolk at the posterior end.

Some eggs were placed half-an-hour after fertilization in solutions of sugar, sodium chloride, and potassium chloride. Four to six hours later, when in the two- and four-cells stages they were put into pure water. Some of the eggs developed normally, but there were also many that developed abnormally. These results seem to show that the effect of the salt-solution persists after the eggs have been removed from it.

Bataillon thinks that the effect of these solutions is physical, and not due to the specific nature of the salts themselves. He found that the eggs that have just reached the uterus behave differently from those that have accumulated there for some time. The former he calls immature. If placed in solutions of these salts, very abnormal development takes place without definite traces of the formation of the embryo or of the process of gastrulation being apparent.

A most thorough and elaborate series of experiments have been made by Madame RONDEAU-LUZEAU to test more especially the action of several chlorides on the development. She finds that a double action exists (contradicting BATAILLON's conclusions on this point), namely, a physical action, due to »hypertonicity« of the solutions, and a chemical action depending on the kind of salt employed. With the exception of lithium chloride the chemical action never predominates if weak solutions are used. The chemical action is masked by the stronger action of the hypertonicity at temperature favorable to development, and above 10 degrees C., but the chemical effect becomes manifest when the temperature is lowered. Before and during fecondation the results are proportional to the molecular concentration. After fecondation the results depend largely on the chloride employed. Except in the case of lithium chloride, the death of the egg is much oftener caused by the osmotic pressure of the solutions. The concentration quickly arrests the development. The teratological action of the chlorides appears to be due to the chemical action in the case of eggs treated after fertilization. The salts act in the following order NaCl, $MgCl^2$, $CaCl^2$, KCl, and LiCl. Between the action of the last two there is a great difference. The effects of the solutions are also more apparent at certain stages than at others, noticeably at the time of closure of the blastopore and of the formation of the medullary folds.

The results that concern us most nearly are those with lithium chloride whose chemical action is much more powerful than that of any of the other salts. Thus it was found that the minimal amount of this salt that will affect the development may be represented by an osmotic pressure of 169 cm (0.3 to 0.4 per cent); while for KCl, it is 405 cm (1.2 per cent); for NaCl, 459 cm (1.0 per cent); for $MgCl^2$, 485 cm (1.3 per cent); and for $CaCl^2$, 484 cm (1.5 per cent).

Further Experiments.

In the hope of obtaining more definite data as to the effect of different salts on the different stages of development I carried out in the present spring of 1903 a very large number of experiments with different salts. In order to determine whether the action depended on the osmotic pressure in the solutions, I put eggs also into solutions of grape and cane sugar that give equivalent osmotic pressures. Although many thousands of eggs were used, and in very different stages, the results were far from satisfactory because the action is too indefinite. Eggs in the same bunch often developed quite differently, although they were in the same stage of development when the experiment began, and were subjected, as far as possible, to the same conditions. Some rather general conclusions may be drawn from these results, but the physiological side of the problem is far from being in a satisfactory condition.

Solutions of lithium chloride of the following strengths were used: 2.5, 3, 4, 4.5, 5, 5.5, 6, 7, 8 per cent. In a number of cases several different solutions were tried at different times, more especially those between .4 and .6 per cent. In a few cases the solutions that had been used for one series were used over again, but this is not to be recommended because an unknown amount of weakening has taken place from the first lot of eggs placed in the solution. Sufficiently large amounts of the solutions were use so that dilution caused by loss of water from the egg-membranes would not produce any great difference in the strength of the solution. Using the .4 per cent solution as a basis, solutions of the following salts were made up; the first of each being nearly equivalent to the 0.4 per cent solution of lithium chloride, the other stronger and nearer the 0.6 per cent solution. The figures give the weight in grams of the substance that was added to 100 cc of spring water.

Lithium iodide	1.5	Calcium chloride	.95
	1.8		1.5
Lithium bromide	—	Sodium chloride	.56
	.81		.8
Lithium nitrate	.63	Cane sugar	4
Lithium benzoate	1.1		6.4
	1.6		8
Lithium salicylate	1.45		10
	2.1	Grape sugar	2
Magnesium chloride	.59		3.2
	8		5
			6

Summary. Despite the large number of eggs used in these experiments those that produced the inversion embryos, shown in our first figures, Figs. *A*—*L* was very small. They occurred in the

.4, .5, .6 per cent solutions, and it was rather noticeable that when this type was found they appeared in lots in which there were generally other eggs like Fig. *D*, but what connection exists between these results I could not ascertain. Moreover I had hoped to find out what the early stages of these inversion eggs are like, but this I was unable to do, because of the rather rare occurence of this type. The other salt in our list that gave also inverted embryos was lithium bromide.

The magnesium chloride solutions, especially the stronger, appeared to have a specific action on the development, especially on the eggs put in while in the two and four cells stages. The later stages developed into tadpoles that lived for many days in the same solutions. I shall hope to describe these magnesium embryos more fully at another time.

The lithium benzoate and salicylate killed the eggs before they developed any further. The lithium iodide was probably too strong, since the development was stopped.

In the stronger sugar solutions the development soon stopped before the embryo appeared. In the weaker solutions the development seemed to be normal, while in the intermediate strengths the overgrowth of the blastopore lips was interferred with so that some spina bifida embryos were produced. There were none of the eggs that showed the inversion condition as in the lithium chloride. The general effect of the sugar solutions was different from that of the lithium salts.

Conclusions. The action of the lithium salts is a specific one, in part at least, and does not simply depend on the physical action of the salt. The salt undoubtedly dissociates, and it is probable that the lithium ion is the effective component. This is indicated by the similar action of lithium bromide on the eggs. Neither magnesium, nor sodium, nor calcium chlorides produced any of these inversion embryos. Comparison with the sugars, which do not dissociate, but act directly through their osmotic pressure on the egg indicate, if they do not prove, that the osmotic pressure is only one of the factors in the salt-solutions. My results have shown that the stage of development at which the eggs are put into the salt-solutions is an important factor in the end-result. We must be on our guard, therefore, in comparing the results of different investigators who may have placed the eggs in the solutions at different times. For this reason I have felt some hesitation in comparing my own results with those of

Hertwig and of Gurwitsch. The egg appears to be much more resistant after the first cleavages have taken place, than during this time, and the gastrula stages are also more resistant than the later segmentation stages. Moreover it is not only a question of resistance, but somewhat different results are obtained according to the stage reached by the egg when it is placed in the solution. The presence of the segmentation cavity in the eggs may be one of the most immediate causes of this difference in the behaviour of younger and of later stages. The shifting of the protoplasm of the upper hemisphere, during the formation of the segmentation cavity, which is interferred with to some extent when the eggs are put into the solutions at an early stage, may also account for some of the differences in different stages.

Summary.

I.

Solutions of 0.4 to 0.6 per cent lithium chloride bring about marked changes in the development of the frog's egg. The results depend, in part, on the the stage at which the eggs are put into the solutions. If put in during the two- and four-cell stages some of the eggs produce embryos similar to those shown in Figs. *Q* and *R*. In these eggs the whole of the black hemisphere sinks into the interior of the egg, as shown in section in Fig. X. An archenteric invagination generally appears at one side in the yolk cells, Fig. VIII. In other eggs the black cells do not sink into the interior, but form a cap over one hemisphere that is sharply marked off by a blastoporic rim from the yolk-cells (Fig. *T*). An archenteron is present on one side of the black hemisphere, Fig. XII, and in some cases a medullary plate, notochord, etc., may appear, Figs. *M* and *N*. The medullary plate may extend to the top of the egg. It would be erroneous to conclude from the position of the medullary plate in these eggs that in the normal egg also the anterior end of the embryo extends to the top of the egg. The two kinds of embryos, the normal and the lithium embryo, are not directly comparable, because the material out of which the embryonic portion develops is differently located in the two cases. This difference of location is due to the lack of a downward movement of the upper protoplasmic contents of the egg, which, in turn, is connected with the obliteration of the segmentation cavity by the upper cells.

If the eggs are put into the solutions at a later stage of segmentation, two general types of larvae are here also produced. One of these types is like that shown in Fig. *P*, in which an upper solid black hemisphere is sharply marked off from a lower yolk-hemisphere. Traces of an embryo may appear on the upper hemisphere, as in the last case; and a backgrowth from the region of the dorsal lip of the blastopore is often formed, Figs. *X*, *W*. The other type is an embryo in which a complete inversion of the germ layers takes place Figs. *A* to *L*. The entire upper part of the egg sinks into the interior Fig. V. Inside the egg this mass of inturned ectodermal cells forms a medullary plate, which is bent back on itself as shown in sections in Figs. I, II, III. A slate-colored area forms a superficial band around the equatorial part of the egg, Figs. *A*, *J*, *L*. Below this the opening of the archenteron appears on the surface. Along the middle of the slate-colored area, where it is broadest, a darker line indicates the position of the notochord, Fig. *B*, which lies just below the surface. Sections show lateral mesoderm, on each side of the notochord, extending around the slate-colored area. A single layer of yolk-cells covers the slate-colored area, having been drawn upwards as the top of the egg sank into the interior, Figs. I to V.

II.

Comparisons with other salts, and with sugar solutions, show that the lithium salts have not only a physical action on the egg, but also a chemical action, due apparently to the lithium.

Zusammenfassung.

I.

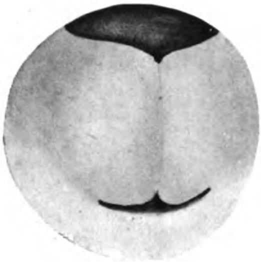
0,4—0,6%ige Lösungen von Lithiumchlorid bringen deutliche Veränderungen in der Entwickelung des Froscheies zuwege. Das Ergebnis hängt theilweise von dem Stadium ab, in dem die Eier in die Lösungen gelangen. Werden sie während des Zwei- und Vierzellenstadiums hineingethan, so ergeben sich Embryonen ähnlich denen in Fig. *Q* und *R*. In diesen Eiern sinkt die ganze schwarze Hemisphäre ins Innere des Eies, wie dies der in Fig. X abgebildete Schnitt zeigt. Eine Urdarmeinstülpung erscheint im Allgemeinen an einer Seite im Bereich der Dotterzellen, Fig. VIII. Bei anderen Eiern sinken die schwarzen Zellen nicht ins Innere, sondern bilden über einer Hemisphäre eine Haube, deren Grenze durch eine Blastoporusspalte scharf von den Dotterzellen abgegrenzt wird (Fig. *T*). Ein Urdarm ist an der einen Seite der schwarzen Hemisphäre vorhanden (Fig. XII) und in manchen Fällen kann eine Medullarplatte, eine Rückensaite etc. erscheinen Fig. *M* und *N*). Die Medullarplatte kann sich bis zum Eischeitel ausdehnen.

Es wäre ein irriger Schluss, aus der Stellung der Medullarplatte bei diesen Eiern zu folgern, dass sich im normalen Ei das Vorderende des Embryo gleichfalls bis zum oberen Eipol erstreckt. Die beiden Arten von Embryonen, normale und Lithiumembryonen, sind nicht direkt vergleichbar, weil das Material für die Entwickelung des embryonalen Antheils in beiden Fällen verschieden lokalisirt ist. An dieser Verschiedenheit ist das Fehlen einer Abwärtsbewegung seitens des oberhalb gelegenen Protoplasmainhaltes des Eies schuld, welche ihrerseits mit dem Verschluss der Furchungshöhle durch die oberen Zellen in Zusammenhang steht.

Gelangen die Eier in einem späteren Stadium in die Lösungen, so entstehen auch hier zwei generell unterschiedene Larventypen. Eine von ihnen ist ähnlich der durch Fig. *P* dargestellten, bei welcher eine solide schwarze Oberhälfte von einer darunter gelegenen Dotterhemisphäre scharf abgegrenzt ist. Spuren eines Embryo können, wie im letzteren Falle, im Bereiche der oberen Hälfte auftreten; auch kommt eine Entwickelung nach rückwärts von der dorsalen Blastoporuslippe öfter zu Stande (Fig. *X*, *W*). Der andere Typus ist ein Embryo, in dem eine völlige Umkehr der Keimblätter Platz greift (Fig. *A*—*L*). Der ganze obere Theil des Eies sinkt ins Innere (Fig. V). Diese nach innen umgewendete Ektodermzellenmasse bildet im Inneren des Eies eine Medullarplatte, welche sich über ihre Fläche zurückbiegt, wie Fig. I, II, III zeigen. Ein schieferfarbiger Bezirk bildet einen oberflächlichen Streifen rund um den äquatorialen Theil des Eies (Fig. *A*, *J*, *L*). Unterhalb desselben erscheint die Öffnung des Archenteron an der Oberfläche. Entlang der Mitte des schieferfarbigen Bezirks, an seiner breitesten Stelle, bezeichnet eine dunklere Linie die Lage der Rückensaite (Fig. *B*), welche unmittelbar unter der Oberfläche liegt. Schnitte zeigen laterale Mesodermentwickelung beiderseits von der Rückensaite in der ganzen Ausdehnung des schieferfarbigen Bezirks. Diesen bedeckt eine einfache Lage von Dotterzellen, die nach oben gelangten, als der Eischeitel ins Innere versank (Fig. I—V).

II.

Vergleiche mit anderen Salzen und mit Zuckerlösungen zeigen, dass die Lithiumsalze nicht allein eine physikalische Wirkung auf das Ei ausüben, sondern auch eine chemische, woran offenbar das Lithium schuld ist.



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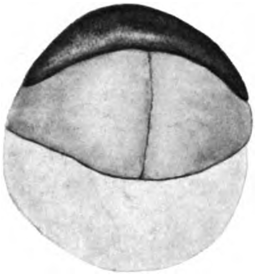

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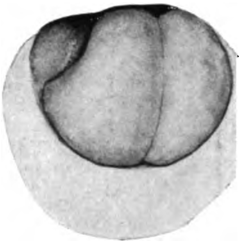

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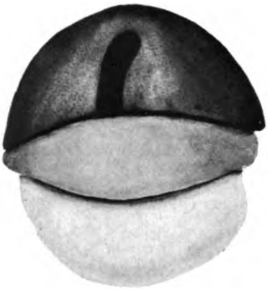

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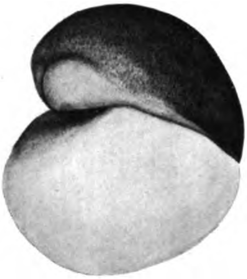

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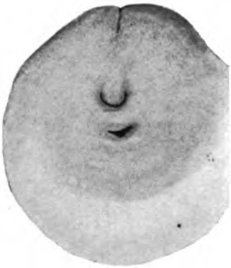

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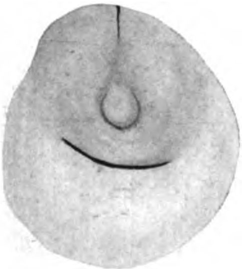

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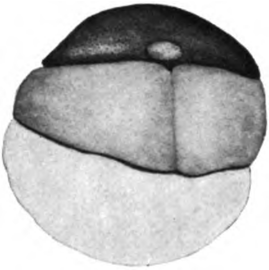

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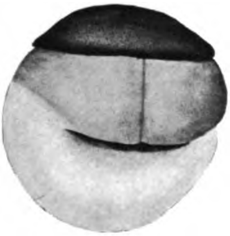

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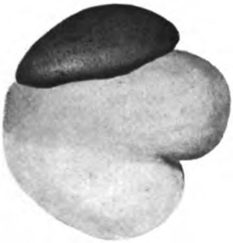

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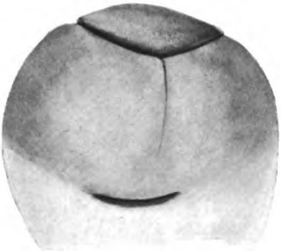

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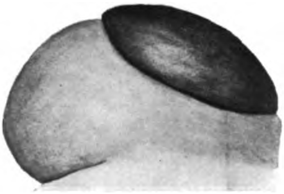

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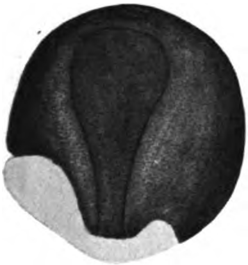

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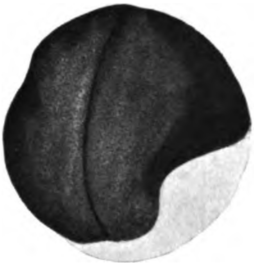

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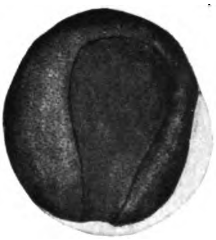

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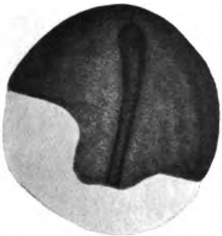

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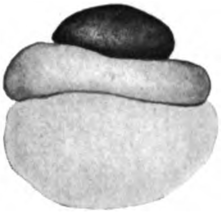

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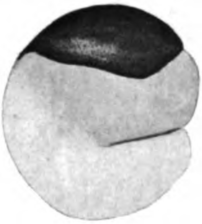

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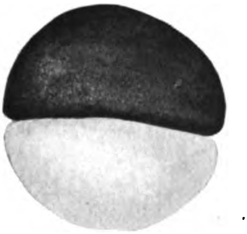

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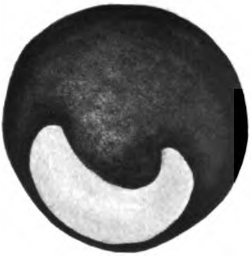

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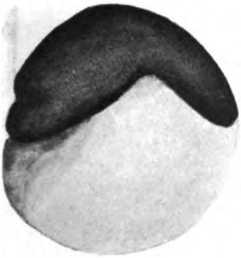

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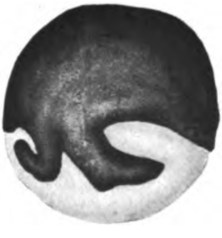

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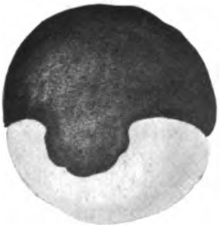

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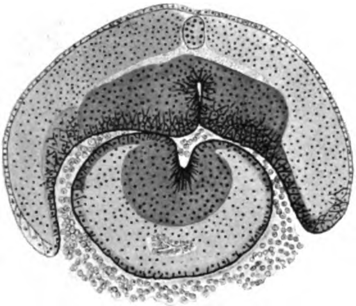
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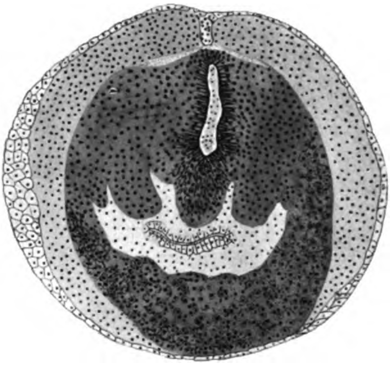
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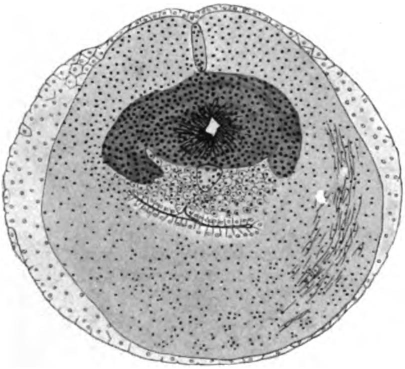
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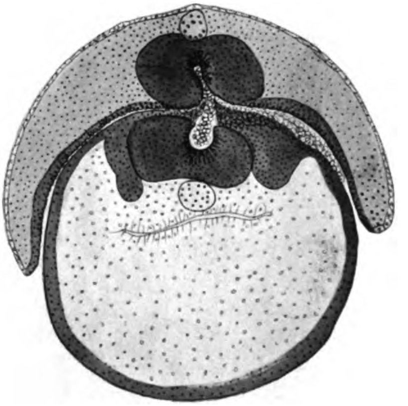
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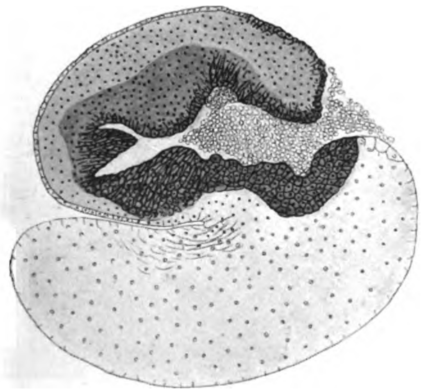
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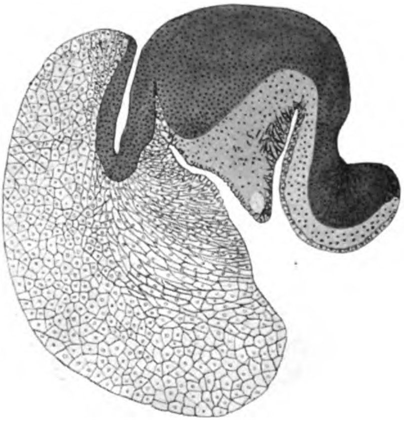
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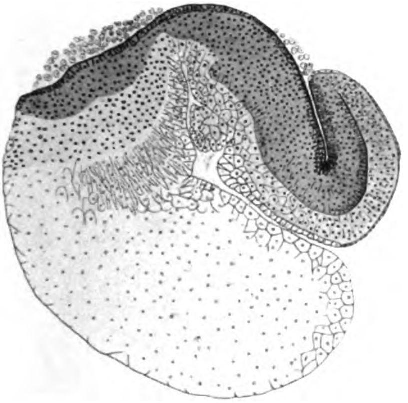

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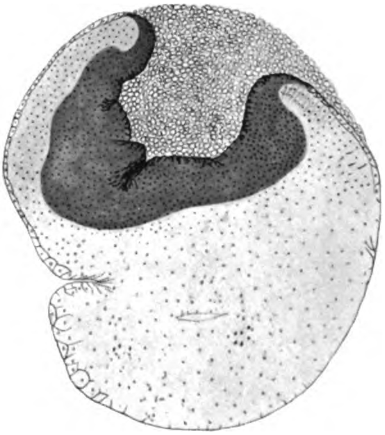

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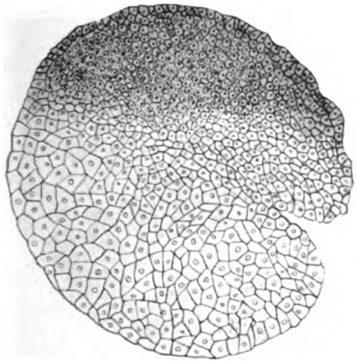

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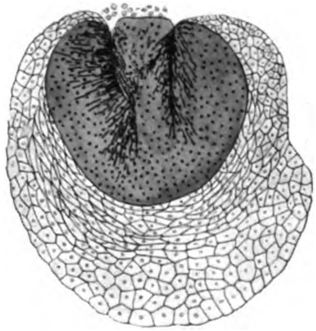

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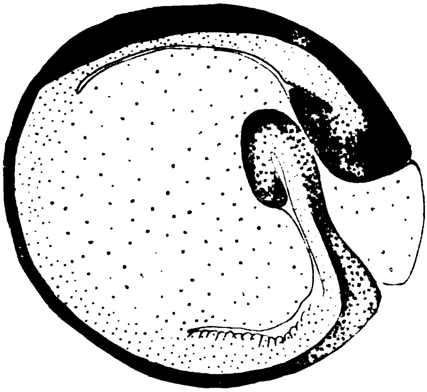

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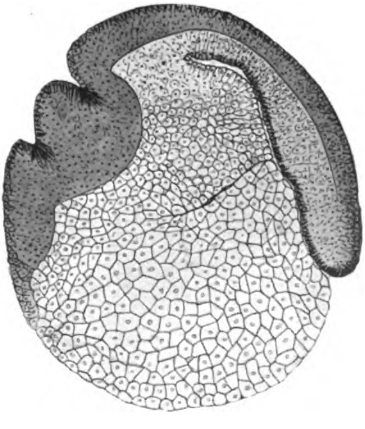

XII

Verlag von **Wilhelm Engelmann** in

Experimental Studies on Eggs of Echinus microtuberculatus.

By

N. M. Stevens.

With Plate XIII.

Eingegangen am 22. Juli 1902.

The following experiments were made at the Naples Zoological Station during December, January, and February, 1901—1902, at the suggestion of Prof. T. H. MORGAN of Bryn Mawr College. The problem was to cut Echinus eggs, in an anaphase of the first division (Fig. 1), into pieces containing less than the normal number of chromosomes; and then to determine whether the less number remains constant in subsequent divisions, or the normal number reappears. DELAGE ('02) claims that the number of chromosomes is constitutional, and therefore the same for embryos from normally fertilized eggs, from fertilized non-nucleated fragments, and from parthenogenetic eggs. If this were the case, the constitutional number should appear in all pieces without regard to the number present when the pieces were cut; but both kinds of sea urchins used by DELAGE, Strongylocentrotus and Echinus, may have thirty-six chromosomes in somatic cells. Probably DELAGE did not count the chromosomes in blastomeres of normally fertilized eggs, and in maturation spindles of eggs from the same individuals as those used in his experiments. In 1890, the number of chromosomes for Echinus microtuberculatus was shown by BOVERI ('90) to be usually 18 in the blastomeres and 9 in maturation spindles: four exceptions were noted, — 18 in one germ vesicle, 18 in one polar spindle, 27 and 23 in the equatorial plate of the first division spindle. In 1902, Prof. BOVERI counted 36 in three cases. At his suggestion I repeated the experiment of fertilizing non-nucleated fragments, and found 18 in every case where it was possible to count with certainty. I then counted in the polar plates of the first and second division spindles of fertilized eggs, and found 18 in eggs from two individuals, and in all others 23 to 36, usually 36. I also counted the chromosomes in maturation spindles from nine individuals, and found in every case 18. The normal number of chromosomes for these experiments must therefore be considered to be 36, with occasional variations between 18 and 36.

Many other points of interest in connection with the problem of cell division, came out in the course of the work, end they will also be discussed.

Method. — The eggs of Echinus are small, and the first division spindle is small in proportion to the size of the egg (Fig. 1), making the problem a difficult one. The method of shaking with pieces of thin cover-glass was tried, but without satisfactory results, the effect of the shaking being to injure the eggs rather than to cut them into small pieces, as it is possible to do with unfertilized eggs.

Finally glass slides were coated on one side with a layer of hard paraffine, the eggs were placed on the paraffine with as much water as would stay on the slide, and were cut into two parts with a sharp lancet needle, under the low power of a compound microscope, Bausch and Lomb, objective 1 in., ocular C, the pieces being left attached to the two sides of the cut in the paraffine. It was impossible to cut the eggs precisely; for, though the orientation of the spindle could be plainly seen, the egg was almost sure to turn under the knife, and the spindle was probably, in many cases, contorted and thrown out of its normal position by the pressure of the knife. On each slide, as many eggs as possible were cut while they remained in a desirable stage, early or late anaphase; and the greatest care was taken to cut only such eggs as were dividing normally. The slides were then placed in a moist chamber for from four to seven hours. When the slides were left over night, the blastulae freed themselves from the paraffine and were swimming about. It was found to be possible to fix the pieces in sublimate-acetic, dehydrate, clear and mount in clove oil, while they were still attached by the egg membrane to the paraffine, and also possible to see with the objects thus mounted what specimens it was worth while to remove with a capillary tube and mount in balsam.

An attempt was made to cut the eggs into several pieces, but in most cases, the pieces resulting from the first cutting were loosened from the paraffine and the smaller pieces would not remain attached. Fig. 11 shows one specimen where a small piece (*a*) was cut off, and the larger piece (*b*) cut partly in two.

I also tried to remove the two halves of several eggs to a watch glass, so as to study their development under the microscope, but without success.

A few eggs were cut before fertilization and then fertilized on the slide. In this way several pieces were obtained, some containing 18 chromosomes in each cell, others 36 (Figs. 5 and 6), the result evidently of fertilization of nucleated and non-nucleated fragments. These pieces did not, as a rule, adhere to the paraffine; in fertilized eggs, the egg membrane holds the piece to the paraffine, as shown in Fig. 16. In the other figures the outline of the membrane is omitted.

The eggs of Echinus were found to be better for these experiments than those of either Sphaerechinus or Strongylocentrotus, as they are somewhat larger and more pliable, yielding more readily to the pressure of the knife.

Results. — The results of the experiments are, of course, only partial or provisional; but if the number of chromosomes in a species is constitutional, I see no reason why the characteristic number should not appear in the first and all subsequent divisions of all pieces containing a centrosome and chromosomes.

Several parts of eggs were found where the number of chromosomes, after several cell divisions, was much less than the normal number. In many cases it was impossible to say that the number counted was uniform for all cells, as only in certain favorable stages is it possible to count, but the size of resting nuclei and the amount of chromatin in them appeared to correspond to the number counted in division stages. Fig. 2 shows a specimen where it was possible to count the same number, six, in approximately half of the cells. Fig. 3 *a*—*e* shows cells from this piece, drawn with higher magnification; and Fig. 4, a cell from the other part of the same egg, in no cell of which was it possible to count, but the spindle indicates at least the full number of chromosomes. If the egg was cut as in Fig. 1 *a*—*a*, the remainder of the chromosomes belonging to the daughter plate which was cut through may have been destroyed instead of being left without a centrosome as in Fig. 9. Fig. 7 is from another piece in which 9 or 10 chromosomes were counted, and in still another piece four were found in several cells. Many pieces containing very small resting nuclei of uniform size were seen, some also with a single vesicle near each centrosome in a metaphase (Fig. 16).

Cell Division. — The first cell division seems to be completed in most cases, regardless of the fact that neither spindle, chromosomes nor centrosome may be present in one or both parts of the piece (Figs. 2, 10*a*, 11*a*, 12*a*). A great many pieces mere noticed where one division was present, as in Fig. 12*a*; other similar pieces had asters in one part, and one piece had asters in both parts. No further cell division occurred when only asters were present. It appeared at first that such divisions had taken place, but very close examination revealed some chromatin wherever cell-division had occurred, a single vesicle (Fig. 16), two or three such with each aster, or very small resting nuclei faintly stained. On this point the results agree with those obtained by Boveri ('96) and Morgan ('99), but differ from those of Ziegler ('98).

Multiplication of Chromosomes in the absence of Centrosomes and Spindle. — Very early in the work, what appeared to be multiplication of chromosomes independently, without the presence of centrosomes or spindle, was observed in the large undivided cells that resulted when one centrosome had been cut away (Fig. 1 *b*—*b* and Figs. 9, 10, 11, 12). The number of chromosomes in such cells was far greater than the normal number; in many cases there was no nuclear membrane, and the chromosomes were somewhat scattered as if ready for mitosis, in others chromatin rings or vesicles were observed (Fig. 11*b* and *c*), and in still others a nuclear membrane was present (Fig. 9*b*). In such in toto preparations, stained with borax carmine, it was impossible to demonstrate the splitting of the chromosomes and separation of the halves, but the large number was very evident; more than fifty were counted in one plane where the nuclear membrane had disappeared and the chromosomes were somewhat separated: The presence of the nuclear membrane in some cases, and its absence in others was also suggestive. According to the belief of Boveri ('88) and Brauer ('93) that the splitting of the chromosomes is an independent reproductive act on the part of the chromosomes themselves, it seems very probable that in these large centrosomeless cells, the nuclear membrane disappears and the chromosomes double in number periodically, just as the centrosomes multiply where no chromatin is present.

Centrosomes de novo. — In some of the large cells described above, especially when the eggs had been allowed to develop longer than usual, centrosomes were observed (Figs. 10*a* and 13), and in a few cases the first or second division was taking place (Figs. 14 and 15). In most of these cases it was certain that the centrosome had been removed, and in others the large amount of chromatin indicated the recent appearance of the centrosomes. Fig. 14 shows a first division spindle in such a cell, and two smaller asters. Fig. 15 showing a very large number of chromosomes, was taken from a specimen where one division had already occurred. Nothing was found in the course of these experiments to indicate that centrosomes had appeared de novo except in connection with chromosomes that had been left by the operation without a centrosome, and in every such case several divisions had occurred at the other end of the half-egg, giving from 40 to 60 cells.

In a few cases the results indicated that the cutting had been done in so late an anaphase that daughter centrosomes had been separated by the knife at one pole of the egg giving one piece consisting of many small cells containing a less number of chromosomes, and another piece having the normal number in the cells of one end, and a less number in those of the other end.

The results of these experiments, though somewhat provisional, have furnished so many points of interest in connection with the whole question of cell division, and the hereditary value of individual chromosomes, that I hope, at some future time to carry the development of such pieces of eggs to a more advanced stage, and also to follow their development with the microscope.

The following enumeration will show some of the variations in the results obtained from individual eggs.

A { 1) Many small cells . . . . Large cell with abnormal no. of chr'mes.
  2) Piece with one division, no chromosomes, no asters.

B { 1) Nothing in one end . . . . Several cells with very small nuclei.
  2) Many small cells . . . . Large cell with abnormal am't of chromatin and radiations.

C { 1) Large cell with very many chromosomes . . . . Many small cells.
  2) Large cell with asters . . . . Large cell with much chromatin.

D { 1) Piece containing only asters.
  2) Piece containing only asters.

E { 1) 30—40 small cells . . . . Large cell with much chromatin.
  2) Large cell with much chromatin . . . . Many small cells.

F 1) Several cells with normal no. of chr'mes.... Large cell with abnormal no.
2) ... Piece with asters.

G 1) Asters .... Many small cells.
2) Piece containing neither asters nor chromatin.

H 1) Several cells containing abnormal am't of chromatin.
2) Piece containing neither chromatin nor asters.

I 1) Many cells with a small no. of chromosomes.
2) Piece containing neither chromatin nor asters.

J 1) Piece with one division, but neither chromatin nor asters.
2) Two large cells with abnormal no. of chr'mes..... Many small cells.

K 1) Piece with one division, but neither chromatin nor asters.
2) Two cells, each containing an abnormal no. of chromosomes.

L 1) Large piece with about twice as many chromosomes in the cells of one half as in those of the other (36, 18).
2) Small piece with about the same number of chromosomes as in one end of the other piece (18).

Conclusions.

1) A part of an Echinus egg, containing a centrosome and a small number of chromosomes (4—12), may go as far as the fifth or sixth division after the operation, without returning to the constitutional number of chromosomes.

2) Chromosomes may divide repeatedly without the presence of a spindle or the occurrence of nuclear or cell division.

3) Centrosomes may appear de novo in a blastomere from which the centrosome has been removed in the anaphase of the first division.

4) The first segmentation division is usually completed in pieces which include the plane of that division, but do not contain the spindle.

5) With the exception of the case cited in (4) cell division in these pieces of Echinus eggs occurred only where chromosomes and centrosomes were present.

Würzburg, Germany, June 20, 1902.

Zusammenfassung.

1) Ein Theil eines Echinus-Eies, welcher ein Centrosoma und eine kleine Anzahl Chromosomen enthält (4—12), kann in der Entwickelung bis zur fünften

oder sechsten Theilung nach der Operation vorschreiten, ohne zu der konstitutionellen Anzahl von Chromosomen zurückzukehren.

2) Chromosomen können sich wiederholt theilen, ohne dass eine Spindel vorhanden zu sein oder Kern- bez. Zelltheilung einzutreten braucht.

3) Centrosomen können ganz von Neuem in einer Blastomere erscheinen, aus der das Centrosom während der Anaphase der ersten Theilung entfernt worden ist.

4) Die erste Furchungstheilung wird von Stücken, welche die entsprechende Theilungsebene einschließen, die Spindel aber nicht enthalten, gewöhnlich vollendet.

5) Mit Ausnahme des unter 4) angeführten Falles trat bei diesen Stücken von Echinus-Eiern nur dann Zelltheilung ein, wenn Chromosomen und Centrosomen gegenwärtig waren.

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Description of Figures.

Plate XIII.

Fig. 1. Somewhat diagrammatic drawing of an Echinus egg to show the relative size of egg and spindle, and various directions in which the eggs may have been cut.

Fig. 2. Part of a cut egg; no nuclei or asters in one end, and only six chromosomes in the cells of the other end. The other part of the egg (not drawn) consisted of many small cells containing approximately the normal number of chromosomes, impossible to count accurately. Counted 36 in blastomeres, and 18 in polar spindles of same lot of eggs. Bausch and Lomb obj. 1.5, oc. C.

Fig. 3 *a*—*e*. Cells from part of egg shown in Fig. 2, higher magnification. Zeiss obj. 1.5, oc. 6.

Fig. 4. Cell from the other part of the same egg. Zeiss obj. 1.5, oc. 6.

Fig. 5 *a*—*b*. Cells from part of an egg cut before fertilization, 18 chromosomes. Zeiss obj. 1.5, oc. 6.

Fig. 6 *a*—*b*. Cells from part of an egg cut before fertilization, 36 chromosomes. Zeiss obj. 1.5, oc. 6.

Fig. 7. Cell from another piece containing nine or ten chromosomes. Zeiss obj. 1.5, oc. 6.

Fig. 8 *a*—*b*. Cells from the two ends of one piece, the other piece having a small number of chromosomes. ZEISS obj. 1.5, oc. 6.

Fig. 9. Two parts of one egg; (*a*) containing only asters; (*b*) only a nucleus — no centrosome — at one end, and many small cells with apparently the full amount of chromatin at the other. ZEISS C, oc. 6.

Fig. 10. Two parts of one egg; (*a*) containing only asters in one end and nothing in the other; (*b*) having a large nucleus with centrosomes in one end and many small cells in the other. ZEISS C, oc. 6.

Fig. 11. Pieces of the same egg; (*a*) containing neither asters nor nuclei, but showing the first division; (*b*) cut partly through as in Fig. 1 *d*, probably destroying one centrosome and leaving two large cells containing chromosomes but no centrosome, while at the other end are many small cells with normal nuclei. ZEISS C, oc. 6. (*c*) Chromatin rings or vesicles from the large cells of (*b*). ZEISS obj. 1.5, oc. 8.

Fig. 12. Two parts of one egg; centrosomes and probably a part of the chromosomes destroyed by the knife. ZEISS C, oc. 6.

Fig. 13. Part of an egg when the centrosome was cut away and more appeared later. ZEISS C, oc. 6.

Fig. 14. Cell similar to the large one in Fig. 10 *a*, showing spindle and additional asters. ZEISS obj. 1.5, oc. 4.

Fig. 15. One of two cells similar to above, where the centrosome was removed, — second division with very large number of chromosomes. ZEISS obj. 1.5, oc. 6.

Fig. 16. Piece containing very little chromatin, a single vesicle with each aster, cell division incomplete at one end. ZEISS C, oc. 6.

Fig. 17. Piece containing asters, and very small nuclei at one end; cell division only when nuclei are present. ZEISS C, oc. 6.

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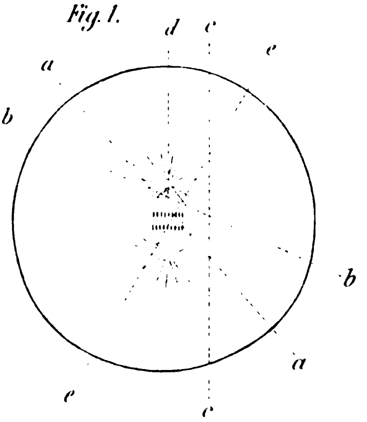

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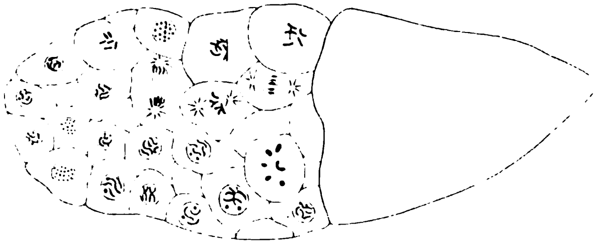

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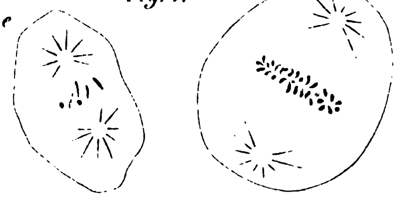

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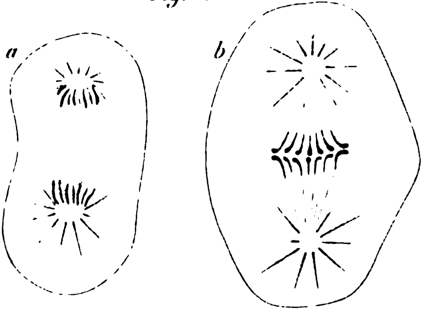

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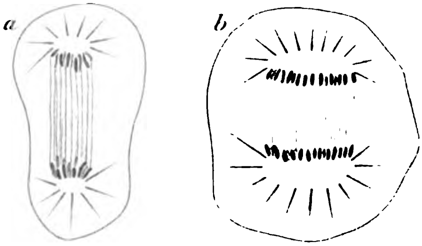

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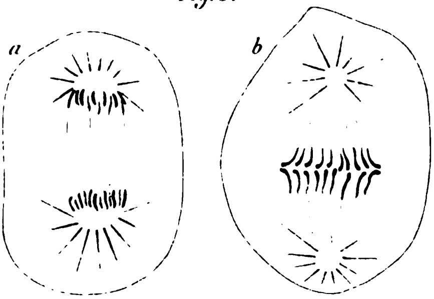

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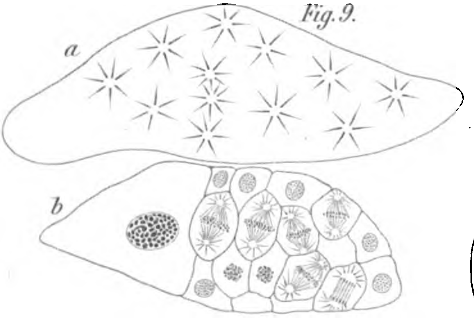

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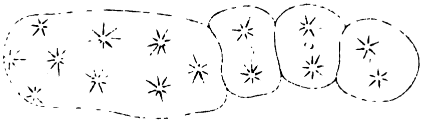

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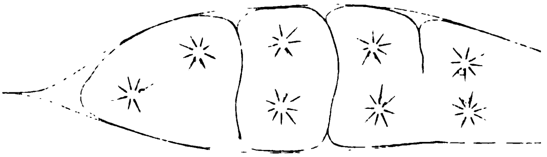

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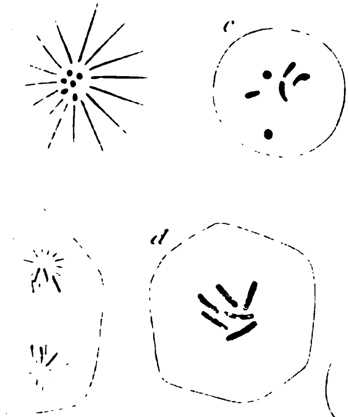

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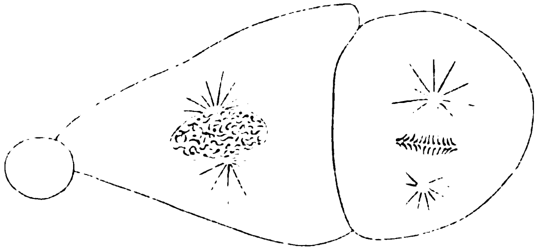

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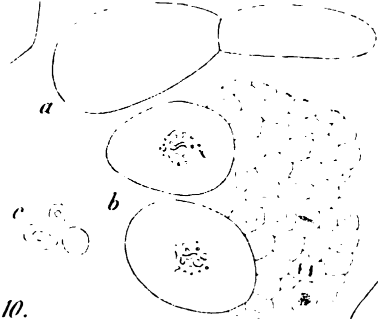

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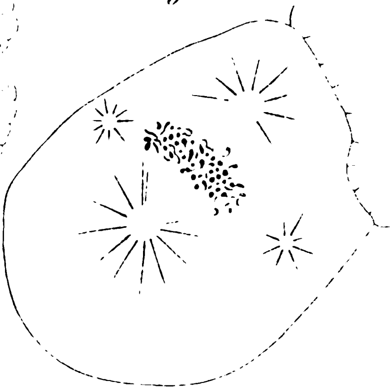

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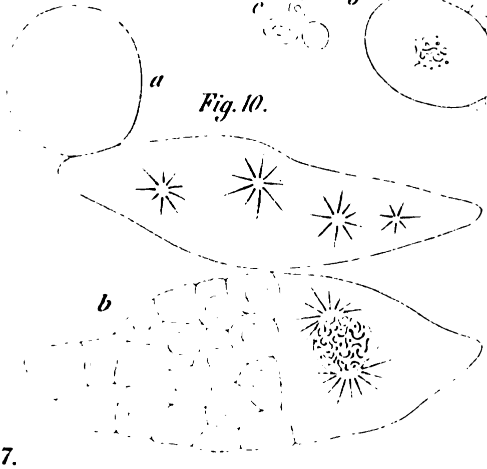

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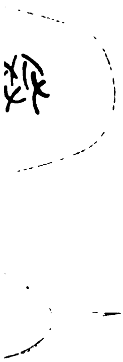

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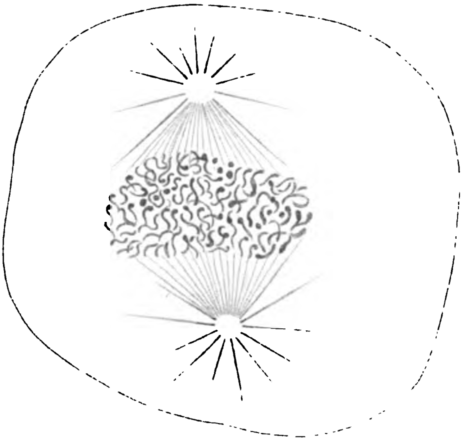

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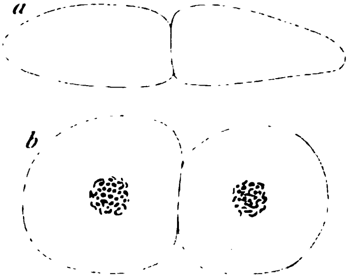

elmann in Leipzig.

Lith. Anst. v. [illegible]

Regeneration in Antennularia ramosa.

By

N. M. Stevens.

With 12 figures in text.

Eingegangen am 22. Juli 1902.

At the suggestion of Prof. T. H. MORGAN, the following experiments were performed at the Naples Zoological Station, during the winter of 1901—1902, Oct. 8th to April 1st. During this period only one colony of Antennularia antennina was obtained, and all the experiments recorded refer to Antennularia ramosa. The habit of growth of the two species is quite different, and this may account for the different results obtained by LOEB ('92), DRIESCH ('97), and MORGAN ('01).

LOEB, working with A. antennina, which has a vertical unbranched stem, found that pieces suspended in sea water always produced roots at the lower end, or lower side if horizontal, and stems from the upper end or side, no matter what the orientation of the piece. He attributed the kind of regeneration that takes place at the two ends or two sides of a piece to the influence of gravity.

A. ramosa, the form used by DRIESCH and MORGAN[1]), is usually branched, the branches often standing nearly at right angles to the main stalk, which itself is not always vertical. DRIESCH found that the basal end of a piece of A. ramosa, when freely exposed to sea water, produced a large number of roots. When the roots were cut off, a few roots were formed and also a delicate stem, which was negatively geotropic. If the end was cut again, generally two or three vigorous stems appeared and rarely a few roots. If these were removed, one or two stems were invariably produced.

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[1]) July 11, 1902. Prof. MORGAN has informed me that the form that he worked on in 1900 was certainly Antennularia ramosa, and not A. antennina, as he then supposed.

Morgan's experiments showed that pieces of different lengths from different parts of the old stem, in nearly every case, produced roots at both ends, without regard to the orientation of the pieces. He also found that pieces remaining attached to stones on the bottom of the tank developed new stems, but no roots; while inverted pieces suspended on the rocks to which they were attached, showed no new growth. He also obtained only negative results from pieces attached to a wheel 30 cm in diameter turned by a stream of water from the tap, and making five and a half revolutions a minute.

My first set of experiments indicated that the region of the stem where the cut was made had more to do in determining the kind of regeneration than polarity, gravity, length or orientation of the piece. The condition of the animals when brought in from the sea is probably another important factor, and one whose influence it is difficult to reckon with. It is possible also that Antennularia may behave somewhat differently at different seasons, though my results obtained in winter do not differ materially from Morgan's in summer.

Methods. — An attempt was made to use the wheel constructed by Morgan for his experiments, but it appeared that the rate of rotation was too great, and that the method of driving the wheel by a stream of water playing on the wheel, — and of course on the pieces of Antennularia, — as well as the unfiltered water in the aquarium where the wheel was placed, were unfavorable. Another wheel, consisting of six blades of cork fastened to spokes projecting from a spindle turned by clockwork, was therefore substituted. This wheel was placed in a tank of filtered sea water and control experiments were carried on in the same tank. The radius of the wheel was 7 cm, and the rate of rotation about one turn in twenty minutes, or about 2.2 cm a minute for any point on the circumference where the specimens were placed.

A large number of experiments were also performed with pieces of various lengths, 2—15 cm, fastened with cactus spines to squares of sheet-cork on glass rods as described by Morgan ('01). The most convenient form was found to be a rod an inch or so longer than the depth of the water in the aquarium, with three or four squares of cork at different levels. This gave space for twelve or sixteen specimens, and could be turned for observation by the part of the rod projecting above water.

Pieces were also suspended by silk threads from bars across the tank, others suspended on the pieces of rock or pottery to which they were attached, still others allowed to sink to the bottom of a glass dish, and pieces of rock with Antennularia attached were placed on the floor of the aquarium. Several experiments were also made with pieces of various lengths, from different parts of the stem, and variously oriented, planted in sea sand at the bottom of a deep battery jar filled with sea water. Pieces with the hydranth bearing pinnae removed were used for a few experiments.

In describing the experiments, the terms apical, median and basal will be used for pieces cut from those parts of the stalk respectively; *d* for distal end, and *p* for proximal end of the piece; roots and stems, as used by DRIESCH and MORGAN instead of the more appropriate, but awkward, technical terms hydrocauli and hydrorhizae.

Pieces attached to cork. — Long and short pieces from all parts of the stalk were fastened to the cork in various positions, — horizontal, vertical, and oblique, distal end uppermost in some cases, in others the proximal, both ends free in the water. A few pieces were placed with one end resting either on a piece of cork or on the floor of the aquarium. The results with some exceptions, as will be seen from the tables and individual records given farther on were as follows: In the majority of cases, a distal or a proximal end from the basal region, below the point where living hydranths are present, produces a stem without regard to orientation; an end from an apical or a median region, roots. When very little is cut from an apical end, not more than two or three joints, the stalk often goes on growing, and no roots are produced, but if a longer piece be cut from this same end, roots will appear.

Basal pieces will usually continue to produce stems, if cut repeatedly, until exhausted by growth or unfavorable conditions. Median and apical pieces will produce roots few or many times according to the vigor of the stalk, and then stems. A long piece which has been cut back two or three millimeters several times, and has repeatedly developed roots and finally stems, will often give roots again, if cut back two or three centimeters. Pieces which are not cut a second time, often produce a second growth of roots or stems, after the first are apparently dead, and, as a rule, finally produce stems last at one or both ends or at the side.

Very often stems or roots or both, are produced at the sides of a piece after both ends are apparently dead. Frequently a stem

Fig. *A*.

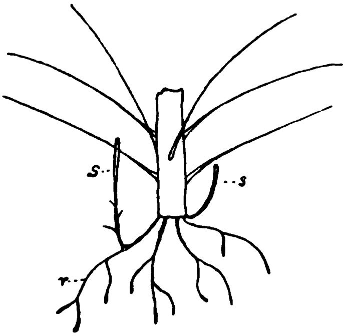


Fig. *B*.

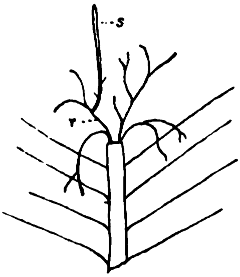


Fig. *A*. Piece of A. ramosa showing new stem (*s*) and roots (*r*) from a proximal end; also a stem (*s*) growing up from a root.
Fig. *B*. Roots and stem from a root at a distal end.

Fig. *C*.

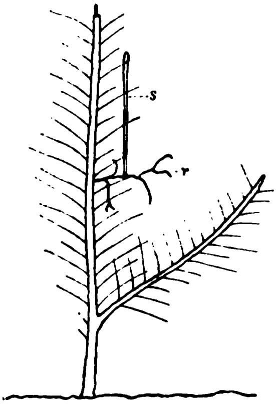


Fig. *D*.

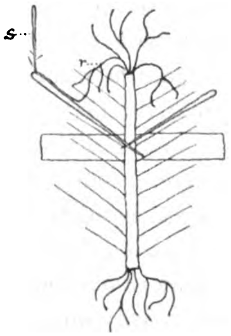
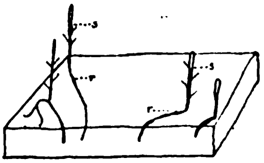


Fig. *C*. Stem from a root at the side of a stalk.
Fig. *D*. Piece of Antennularia attached to cork by cactus spines; roots at both ends, and a stem (*s*) from a root that had run out one of the cactus spines.

develops from a root free in the water, at an upper or lower end or the side of a piece, or from a root which has run out on the cork or on a cactus spine (Figs. *A*—*E*). Such stems have been observed growing on the cork weeks after the parent stalk had been removed; here the so-called roots seem to have the value of stolons in plants. Pieces which were placed with one end resting on cork or on the floor of the aquarium, attached themselves by roots at that end, and produced roots or stems at the other end according as the end was cut from one or another part of the original stem. In several cases a new stem, on coming near to the glass rod, broke up into roots and attached itself to the rod. In a few other cases, what were very evidently stems at first, later divided up into roots; this occurred at both the upper and the lower end of the piece.

The ease with which roots produced stems, and new stems, roots, suggested the desirability of investigating the histological structure of these new growths. Pieces with very vigorous roots and stems 1 to 1.5 cm long, the first regeneration, were fixed in concentrated sublimate solution with 5 % formalin, and the sections stained with Delafield's haematoxylin differentiated with picric acid in absolute alcohol. These sections showed the structure of roots and stems to be identical so far as ectoderm and entoderm are concerned, the only difference being, that the stems usually contain a greater number of tubes than the roots. The cells and nuclei are very similar to those of Tubularia; but the ectoderm and entoderm cells are more nearly alike, and the reserve food granules are in groups between the cells instead of in the endoderm cells as in Tubularia. No cells in mitosis were found in new stems, roots, or in the adjacent end of the old stalk, a point which has an important bearing on the method of regeneration, as will appear later.

Fig. *E.*



Stems growing from roots left on the cork when an old horizontal piece was removed.

The following table shows the first new growth at the cut ends of material brought in at various times; some lots much more vigorous and healthy than others, some in reproductive stage, others not. Some lived for five or six weeks in the aquarium, others only for a few days. The results are, therefore, not so uniform as they would have been with carefully selected material; they do, however, indicate the general rule stated above, which comes out more clearly in later experiments with uniformly good material, on the wheel.

Pieces on Cork.

	Roots at *d*; roots at *p*	Roots at *d*; 0 at *p*	Roots at *d*; stem at *p*	Stem at *d*; stem at *p*	Stem at *d*; 0 at *p*	Stem at *d*; roots at *p*	0 at *d*; roots at *p*	0 at *d*; stem at *p*	Rt. & st. at *d*; rt. & st. at *p*	Rt. & st. at *d*; 0 at *p*	Rt. & st. at *d*; stem at *p*	Rt. & st. at *d*; roots at *p*	0 at *d*; rt. & st. at *p*	Stem at *d*; rt. & st. at *p*	Root at *d*; rt. & st. at *p*	Total
Apical, vert., *d* up	17					8	13									38
- - *p* -	7		1			1	2	2				1		1	1	16
- obl., *d* -						1	1									2
- - *p* -												2				2
- horizontal	1			1												2
Median, vert., *d* up	22		2	1	2	7	6	1		1						42
- - *p* -	18		2	1		1	2	3						1	3	31
- obl., *d* -						2	2									4
- - *p* -	1						2								1	4
- horizontal	2															2
Basal, vert., *d* up	1			23	9	7	3	6			1		1	1		52
- - *p* -			2	18				9		2				1	2	34
- obl. *d* -				1	2	1		1								5
- - *p* -				2		1		2								5
- horizontal			2	4	1			2								9
Basal to median	1		3			1		2							3	10
- - apical			1	1				1			1		1		3	8
Total	70	0	13	52	14	30	31	29	0	3	2	3	2	4	13	266

The following records of individual cases will give a more correct idea of the usual course of regeneration, and of exceptions to the rule.

I.

Oct. 31.	Apical piece, 5 cm, horizontal.
Nov. 3.	Roots at both ends.
- 5.	Cut *p*.
- 9.	Roots again at *p*.
- 13.	Cut *p*.
- 24.	Stem at *p*.
Dec. 1.	Ends dead, but stem at the side and on a root which had run out on the cork.

II.

Nov. 9.	Apical piece, vertical, *p* up, 5.5 cm.
- 13.	Roots at *d*; stem and roots at *p*.
- 24.	- - - roots at *p* (stem broken up into roots).
- 26.	Cut both ends.
Dec. 1.	Nothing at *d*; stem at *p*.
- 16.	- - - 2 stems at *p*.

III.

Nov. 9.	Apical piece, vertical, *p* up, 5.5 cm.
- 13.	Stem at *d*; roots at *p*.
- 18.	Roots at *d*; roots at *p* (stem at *d* broken up into roots).
- 26.	Roots at *d* dead; roots at *p*.
Dec. 9.	Stem at *d*; roots and stem at *p*; stem on one root and on several pinnae at side.

IV.

Oct. 31.	Median piece, horizontal, 5 cm.
Nov. 3.	Roots at both ends; cut *p*.
- 5.	- - - - -
- 9.	- - - - - cut *p* again.
- 13.	- - - - - - -
- 18.	Two stems at *p*.
- 24.	Roots and stem at *d*; stems at *p*.
Dec. 1.	Roots and stem at *d* dead; stem growing at *p*.

V.

Dec. 31.	Median, vertical, *p* up, 4 cm.
Jan. 3.	Roots at both ends.
- 14.	Roots dying.
- 19.	Stem growing up from *d*.
- 26.	Stem at *d* dead; two stems at *p*.
Feb. 2.	Only one stem growing at *p*.

VI.

Dec. 31.	Median, vertical, *d* up, 5 cm.
Jan. 3.	Roots at both ends.
- 7.	Cut *p*.
- 11.	Roots again at *p*.
- 14.	Roots dying at both ends, but new ones coming out.
- 19.	Stem and roots at *p*; roots at *d*.
- 26.	- - - - - - - - -
Feb. 2.	Roots and stems dead at both ends.

VII.

Dec. 31.	Median, *p* up, 4 cm.
Jan. 3.	Roots at both ends.
- 5.	Two stems also at *p*.
- 7.	Cut *p*.
- 11.	Stem at *p*.
- 26.	Roots dead at *d*; stems dead at *p* but two new stems growing.
Feb. 2.	Stems at the side growing, others dead.

VIII.

Dec. 31.	Basal, median, 9 cm, horizontal.
Jan. 4.	Roots at *d*; stem at *p*.
- 7.	Cut both ends.
- 11.	Stem at each end.
- 14.	Stem at *p* growing most rapidly.
- 26.	Stem at *p* dying and new stem growing.
Feb. 2.	Stem at *p* growing, also stem at side.
- 15.	Dead.

IX.

Oct. 31.	Basal, *p* up, 5 cm.
Nov. 3.	0 at *d*; stem at *p*.
- 9.	Cut *d*.
- 13.	Stem at *d*.
- 15.	Cut both ends.
- 18.	Stem at both ends.
- 26.	Stems dead at both ends.
Dec. 1.	New stem at *p* and stems at side.

X.

Nov. 9.	Basal, median, one branch, *d* up.
- 13.	Roots at median ends.
- 15.	Stem at side near *p*. Cut *p*.
- 18.	Roots at median ends dying and stems coming out; stem at *p*.
- 26.	Stem at *p* broken up into roots; stems at median ends growing.

The breaking up of a stem into roots at the lower end of the piece in specimens III and X might suggest the influence of gravity, had not the same thing occurred at the upper end in number II.

Pieces on the wheel. — The following table, and records for several individual cases will serve to show that similar results are obtained when the element of gravity is eliminated. The specimens used in the 2nd experiment were especially vigorous and were cut many times as appears in the records that follow the table. A few pieces on the cork and glass rod were inverted several times a day without any evident effect on the kind of regeneration that resulted.

	Roots at *d*; roots at *p*	Roots at *d*; stem at *p*	Stem at *d*; stem at *p*	Stem at *d*; roots at *p*	0 at *d*; roots at *p*	Total
Oct. 17th						
Apical	6			1	1	8
Median	1			1		2
Basal			4	2		6
Total	7		4	4	1	16
Oct. 31st						
Apical	2			2[1]		4
Median	4					4
Basal		1[2]	3			4
Total	6	1	3	2		12

I.

Oct. 31.	Apical, 5 cm.
Nov. 3.	Roots at both ends.
- 5.	Cut *d*.
- 9.	Roots at *d* again; cut *d*.
- 13.	- - - -
- 15.	Cut *d*.
- 19.	Roots at *d* again; cut *d*.
Dec. 1.	Stem at *d*.

II.

Oct. 31.	Apical, 6 cm.
Nov. 3.	Roots at both ends.
- 5.	Cut *p*.
- 9.	Stem at *p*; cut *p*.
- 13.	Stem at *p*.
- 15.	Cut *p*.
- 19.	Stem at *p*; cut *p*.
- 24.	Stem at *p*.
- 26.	Cut *p*. 1 cm.
Dec. 1.	Stem at *p*; but nearly exhausted.

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[1] Very little cut from the end. See III.

[2] Cut in median region at *d*. See VIII.

III.

Oct. 31.	Apical, 6 cm, *d* cut only a few joints.
Nov. 3.	Stem at *d*; roots at *p*.
- 5.	Cut *d* .5 cm.
- 9.	Stem at *d*; cut *d* 1 cm.
- 13.	Roots at *d*.
- 15.	Cut *d*.
- 19.	Roots at *d*; cut *d*.
- 23.	- - - - -
Dec. 1.	Stem at *d*; piece very short and ap. about exhausted.

IV.

Oct. 31.	Median, 6 cm.
Nov. 3.	Roots at both ends.
- 5.	Cut both.
- 9.	Roots at *d*; stem at *p*; cut both.
- 15.	0 at *d*; stem at *p*; cut both.
- 20.	Roots at *d*; stem at *p*.
Dec. 1.	Roots dead; stem at both ends.

V.

Oct. 31.	Median, 6 cm.
Nov. 3.	Roots at both ends.
- 5.	Cut both.
- 9.	Roots at both ends; cut both.
- 13.	Roots at *d*; stems at *p*.
- 15.	Cut both ends 1 cm.
- 20.	Roots at both ends.
- 23.	Stem also at *p*.
- 26.	Roots dying; cut both ends.
Dec. 1.	Stem at both ends.

VI.

Oct. 31.	Median, 6 cm.
Nov. 3.	Roots at both ends.
- 9.	Cut *p*.
- 13.	Stems at *p*.
- 15.	Cut *p* 1 cm.
- 23.	Roots at *p*, cut *p*.
- 26.	- - -
Dec. 1.	Dead, apparently.

VII.

Oct. 31.	Basal, 5 cm.
Nov. 3.	Stem at both ends.
- 9.	Cut both.
- 13.	Stem at both ends.
- 15.	Cut both.
- 26.	Stem at both ends; cut both.
Dec. 1.	Stem at both ends.

VIII.

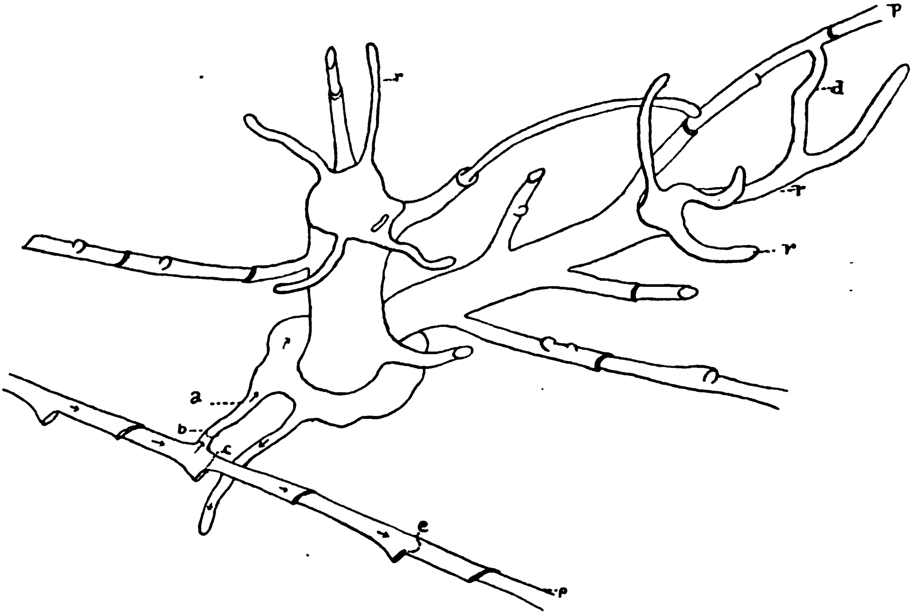
Oct. 31.	Basal, 5.5 cm, *d* in median region.
Nov. 3.	Roots at *d*; stem at *p*.
- 9.	Cut both.
- 13.	Stem at both ends.
- 15.	Cut both.
- 26.	Stem at both ends; cut both.
Dec. 1.	Stem at both ends.

This lot of Oct. 31st was the best material of the winter, grew most vigorously and with almost no unexpected variations. The tendency to produce stems from basal ends, and roots at first, stems later, from median and apical ends, unless cut within the growing region at the apex of the stalk, is very evident. Numbers V and VI illustrate well the point that an end that has reached the condition where it produces a stem instead of roots may be made to produce roots again by cutting back into a region which has perhaps not yet been drawn upon in producing new roots and stems.

Pieces on the bottom of a glass dish. — At various times pieces of different lengths, mostly short, from different regions of the stalk, were thrown into a glass dish and allowed to fall as it happened, basal pieces always horizontal, others generally oblique and sometimes one end up, sometimes the other. Of 11 basal pieces, of which a record was made, 4 produced a stem at both ends; 2 a stem at *d* and nothing at *p*; 5 a stem at *p* and nothing at *d*.

Of 10 apical and median pieces, 6 produced roots at both ends, 3 roots at *d* on the bottom of the dish and stem at *p*; 1 roots at *p* on the bottom of the dish, nothing at *d*. The end which rested on the bottom usually attaches itself to the glass by means of a circular plate of coenosarc, and then sent out roots from the plate and also from the end of the stalk, as well as from the other end. Some of these pieces which produced roots at both ends were very short, not more than .5 cm, others 2—3 cm.

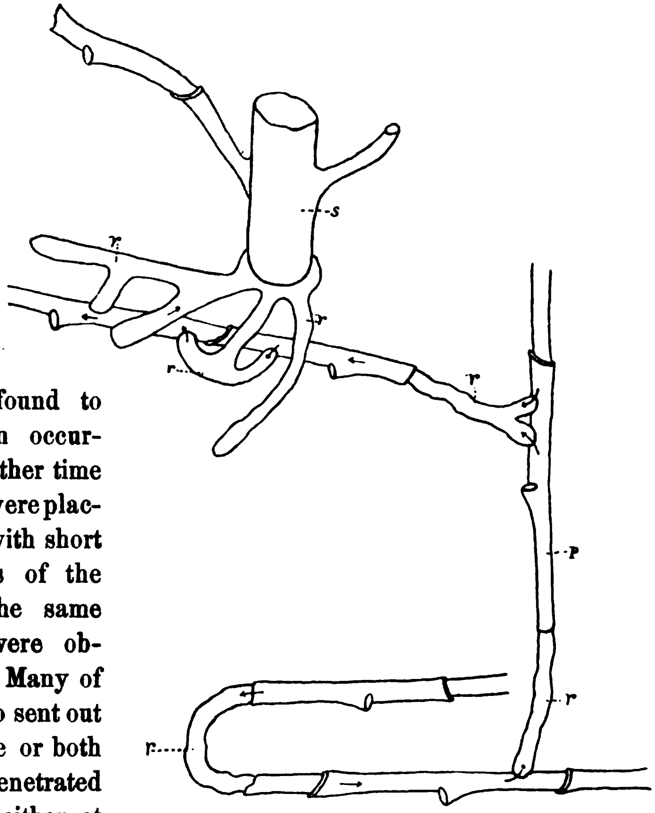
Fig. *F*.



A short piece with one branch, showing union, of a root (*d*) with a pinna (*p*) belonging to the stalk; and union of another root (*a*) with a free pinna (*p*). *b*, *c*, *e* indicate points where a wall of perisarc was built while the specimen was under observation. The arrows indicate the direction taken by the circulating fluid at one time. The direction changes periodically.

One curious case was observed, where a very short piece had fastened itself to the bottom of the dish, and one root had penetrated a pinna near by, not at a hydrotheca, but on the opposite side. The coenosarc of the two, root and pinna, had united and circulation was going on between the pinna and the stalk to which it may or may not originally have belonged (Fig. *F*). The conditions indicated in the figure were observed on the fourth day after the pieces were cut. Four days later nearly all of the coenosarc from the pinna had been withdrawn into the main stalk and a wall of perisarc built across the root *a* at *b*. The coenosarc from the other roots and pinnae was also mostly withdrawn. This withdrawal of coenosarc leaving apparently dead roots and pinnae was found to be of common occurrence. At another time many pinnae were placed in a dish with short median pieces of the stalk, and the same phenomena were observed again. Many of the pinnae also sent out roots from one or both ends, which penetrated other pinnae either at the end or side, and circulation went on between two, three or even four pinnae (Fig. *G*). Evidently the coenosarc has power not only to secrete the perisarc, but to dissolve it as well. The small pieces described above were kept under observation for ten days or more in a covered glass dish containing not more than half a liter of water, which was not changed. In fact, the pieces lived and grew longer if not disturbed by changing the water.

Fig. *G*.



A short piece connected by its roots with a free pinna at four points; also union of four pinnae by roots and circulation through the old stalk, roots and four pinnae as indicated by the arrows.

An interesting result of the study of small pieces under the compound microscope was the discovery that the apparently dead roots and stems on pieces used in other experiments, were really the empty perisarc, from which the coenosarc had been withdrawn into the main stalk. The same phenomenon was observed in pinnae with hydranths; the hydranths were withdrawn from the hydrothecae, and then the whole living contents of the pinnae into the main stalk. This seems to have been the condition in several pieces that had fallen to the bottom of the aquarium and lain there apparently dead for several weeks, but late in March began to send up stems, and were found to be firmly attached to the floor of the tank by roots from the side of the stalk. The same reappearance of life was observed in pieces on rocks at the bottom of the tank.

Pieces suspended in the aquarium by silk thread. — Fearing that contact with cork or cactus spines might affect the regeneration of pieces so attached, I suspended other similar pieces as control experiments, by means of silk thread loosely tied between the pinnae, with the following results:

1) Long piece, median, *p* up, *d* not cut.
Roots at *p*; 0 at *d*.
2) Long piece with 3 branches, median, *p* up.
Roots at all five cut ends.
3) Long piece, median-apical, 3 branches, *p* up.
Roots at all five ends.
4) Median piece, 10 cm, *p* up.
Roots at both ends.
5) Median, 9 cm, *d* up, one branch.
Roots at all three ends.
6) Median-apical, 9 cm, horizontal.
Roots at *p* and lower side; 0 at *d*.
7) Basal, horizontal.
Stem at *p*; 0 at *d*.
8) Basal-apical, 15 cm, horizontal, one branch.
Roots at distal ends; 0 at *p*.
9) Basal-median, oblique, *p* uppermost.
Stem at *p*; roots at *d*.
10) Basal to median, *p* up.
Roots at both ends.
11) Basal to apical, *p* up, 2 branches.
Roots at 3 distal ends; stem at *p*.

12) Basal-apical, *d* up.

Stem and roots at *p*; 0 at *d*.

13) Basal-apical, *p* up.

Stem at *p*; 0 at apical ends.

Many other pieces were suspended, some of them cut several times, and the results agreed with those for the same material attached to pieces of cork.

Inverted pieces suspended on rocks, etc. — Of such pieces only a few, and those cut well back into the basal region showed any sign of regeneration. Seven pieces sent out short stems, but only one, developed a stem which turned around and grew upward, producing pinnae and hydranths. Fig. *H* is a sketch of a suspended piece of rock, on which six pieces produced short stems, three of the pieces projecting upward and three downward. Those that grew downward did not reach a length of more than half a centimeter. Figs. *I* and *J* show a piece which produced a very satisfactory new stem *s*. The piece was cut and placed in the aquarium Jan. 11th, and the drawing, Fig. *I*, made Jan. 14th.

Fig. *H*.

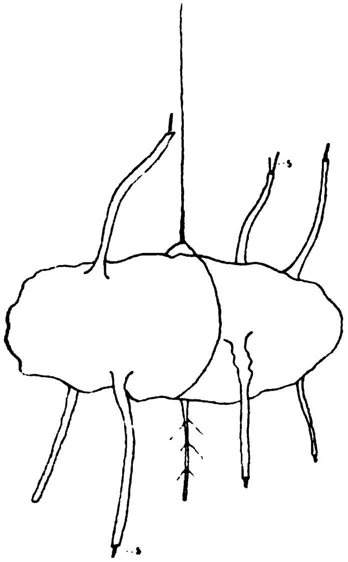


Fig. *I*.

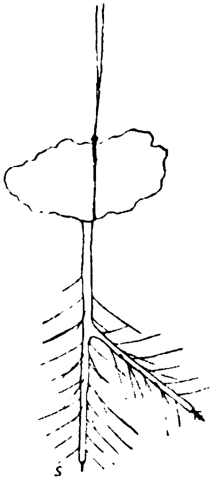


Fig. *J*.

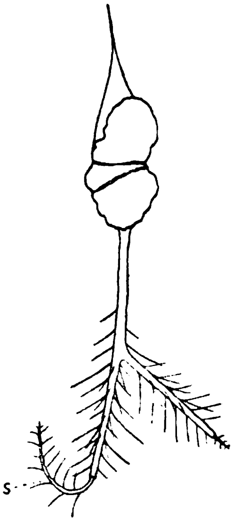


Fig. *H*. Piece of rock suspended so that three stalks of Antennularia were naturally oriented and three reversed, all growing stems.

Figs. *I* and *J*. Piece of A. ramosa suspended by the sand held together by its roots. Fig. *I* after five days. Fig. *J* after fifteen days.

The silk broke, and the specimen was suspended a second time, on Jan. 14th, as seen in Fig. *J*, the latter sketch being made on Jan. 26th. Morgan's results under these conditions were entirely negative, and these experiments show regeneration of attached specimens of Antennularia ramosa with reversed orientation to be of rather rare occurrence.

Pieces on rocks on the floor of the aquarium. — With one exception, pieces left on the rocks in their natural position, never produced roots, and stems only when cut in the basal region. Such new stems often continued to grow for several weeks, reaching a length of several centimeters. The suggestion of Morgan ('01) in connection with a similar experiment, that the presence of roots at one end may have prevented the development of roots at the other end, is not supported by the preceding experiments, in which roots grew a second time at the cut end while roots were present at the other end, either free in the water, or attached to cork or to the bottom of the tank. After obtaining only stems in this set of experiments for five months, on March 22nd, a piece of rock was brought in having attached to it four vigorous stalks of Antennularia, which I cut off at various levels leaving the attached stalks 1, 2, 4, 6 cm in length respectively. The results were as follows:

1) Cut in median region, 6 cm, March 25th, 3 roots.
2) - - - - 4 - - - 0.
3) - - basal - 2 - - - stem.
4) - - - - 1 - - - stem.

The observations of March 25th were confirmed in the following days, but no opportunity for further experiment occurred.

Pieces planted in sand. — At Prof. Morgan's suggestion, pieces of Antennularia, cut as in the other experiments, were planted in sand at the bottom of a deep battery jar so that the upper ends of the pieces were at least 5 cm below the surface of the water.

Out of five experiments, three gave results agreeing approximately with the general rule stated above, while the pieces used in the other two experiments produced only stems. The following is the record of these experiments.

I.

Nov. 21st.
1) Apical, *p* up, roots.
2) - *d* - continuation of stem.
3) - *d* - - - -
4) Median, *d* up, roots.
5) - *d* - -
6) - *d* - -
7) - *d* - stem and roots.
8) Basal, *d* up, stem.
9) - *d* - -
10) - *p* - -
11) - *p* - -
12) - *d* - -
13) - *p* - -

IV.

Jan. 22. Only three pieces grew.
Median, *p* up, stems.

V.

Feb. 20. Eleven pieces of which only seven grew.
1) Apical, *d* up, roots.
2) Median, *d* up, roots.
3) - *p* - -
4) - *p* - -
5) - *p* - -
6) Basal, *d* up, stem.
7) - *p* - -

II.

Dec. 9. Several pieces, apical, basal, and median, *d* up, and *p* up, all grew stems. No reason was evident unless that the material was in less vigorous condition. It was in a reproductive stage and somewhat paler than that of Nov. 21st.

III.

Dec. 31. Also in reproductive stage.
1) Apical, *d* up, stem.
2) - *d* - - and roots at side.
3) - *d* - roots.
4) Median, *d* up, stem and roots.
5) - *d* - roots and stem from one root.
6) Median, *p* up, roots.
7) - *p* - -
8) - *p* - - and stem.
9) - *d* - - Jan. 11. Broken over at surface of sand, roots and stem at break, roots attached to sand, stem growing upward (Fig. *L*).
10) Median, *d* up, roots and stem cut Jan. 7th; no further growth.
11) Basal, *d* up, stems.
12) - *d* - -

In this material a greater tendency to produce stems than in the first experiment may be observed, but not so great as in Exp. II.

The pieces used in the first experiment were all carefully removed from the sand Nov. 30th, after nine days, and no trace of any growth below or at the surface of the sand observed. Moreover, all of the stalk below the surface was in every case merely the empty perisarc, and later investigation showed that by the third day the coenosarc had withdrawn from the tubes below the surface of the sand. In only two cases was any growth discovered at the lower end; one noted above where a stalk, which had fallen over, produced roots and a stem at the broken proximal end (Fig. *L*), and another where roots were sent out from the ends of pinnae above the sand (Fig. *K*).

Pieces with pinnae removed. — Having observed that pieces with broken pinnae and no living hydranths show a tendency to produce stems at any level, also that the basal stem-producing pieces have no living hydranths, and that the coenosarc has been withdrawn from the pinnae of median pieces when, after having been cut several times, they change from the production of roots to that of stems, I thought that the presence of living hydranths might be a factor affecting the kind of regeneration. To test this possibility, the pinnae with hydranths were all cut away from several median pieces before they were attached to the pieces of cork, but these specimens all produced roots at both ends, as usual.

Fig. *K*.

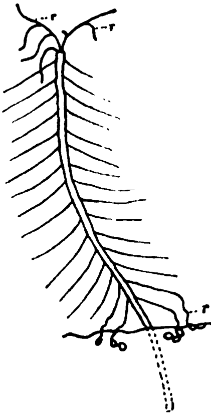


Fig. *L*.

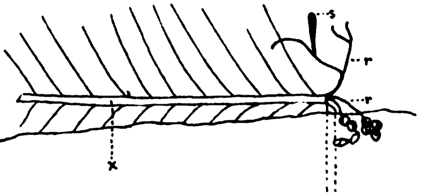


Fig. *K*. Piece planted in sand. Roots growing from ends of pinnae and attaching themselves to sand.
Fig. *L*. Piece planted in sand and fallen over. Roots and stem at the broken proximal end. Coenosarc withdrawn from the other end to *x*.

General discussion. — These experiments show conclusively that in Antennularia ramosa the kind of regeneration at cut ends freely exposed to sea water cannot be determined by the polarity of the piece or by its orientation with respect to gravity. That the putting forth of roots or stems is not a response to the stimulus given by the instrument in cutting, or by the sea water on the cut surface, is shown by the fact that no new growth more than the closing over of the cut end is evident for 48 hours or more, and that the coenosarc is often withdrawn from one set of roots or stems, only to he pushed forth again in new ones, at once, or weeks after the pieces were cut.

That the kind of regeneration does vary with the part of the stalk where the cut is made, but is modified by the condition in which the stalk is at the time when it is cut, is evident. The more brittle median and apical parts of the stalk, which in their natural habitat would most often be broken off and fall upon the rocks in various positions, show a strong tendency to produce roots at any point, while the tougher, less easily broken basal portions usually produce nothing but stems.

Production of stems seems also to be characteristic of an exhausted condition of the organism, not so much a »Verbesserung«, as DRIESCH calls it, as a final effort to prolong its existence, when all attempts to establish normal conditions of attachment have failed.

The entire absence of dividing cells in new growths and in the adjacent end of the old stalk indicate that regeneration in Antennularia does not necessarily involve the production of new tissue, at least at the point where regeneration occurs, but is merely a pushing out from the old stalk of already formed coenosarc. That this is the case is also indicated by the fact that, when an end that has produced roots several times, and develops only a rather sickly stem or nothing at all, is cut back two or three centimeters, it again sends forth vigorous roots in large numbers.

The coenosarc appears to be a very uniform and indifferent structure, capable of pushing out from the parent stalk at any point, in any one of its several forms, — either a plate or root by which to attach itself, or a stem to produce feeding hydranths and thus prolong the existence of the organism. It is also capable of withdrawing itself from parts of the perisarc where conditions are unfavorable; e. g., parts of the stalk below the surface of sand, and roots which have not succeeded in attaching themselves, and it may then at the same or another part of the stalk put forth the same or a different kind of growth.

I take this opportunity to express my indebtedness to Prof. T. H. MORGAN who suggested this work on Antennularia; to the Association for Maintaining the American Woman's Table at the Naples Zoological Station, for the use of the table for six months; to Dr. LOBIANCO for the material used; and to Dr. DOHRN, the professors and attendants at the Station for their uniform kindness during my stay there.

Conclusions.

1) The kind of regeneration at a cut end of Antennularia ramosa is not determined by polarity, or by orientation of the piece with respect to gravity, or by conditions existing at the other end of the piece, unless it be in case of normal attachment.

2) Certain parts of the stalk tend to produce roots, others stems. Basal pieces usually produce stems; median pieces, roots; and apical pieces, cut within the region of growth, tend to continue the stem.

3) A piece which has not succeeded in attaching itself by its roots, or has been exhausted by repeated production of roots, finally produces a stem or stems from the ends or side.

4) The coenosarc of Antennularia ramosa is of such an indifferent character that it may be withdrawn from one form of growth and put forth in another form without the production of new tissue.

5) Regeneration in A. ramosa appears, at least in early stages, to be adaptation of the already formed coenosarc to new conditions and needs, rather than to involve the building of new parts by cell multiplication.

6) The coenosarc may be withdrawn into the old stalk, from all the pinnae and new growths remain dormant for weeks, — two months at least, — and then rapidly push forth new roots and stems at the sides or ends of the stalk.

Würzburg, July 9, 1902.

Zusammenfassung.

1) Die Art und Weise der Regeneration am abgeschnittenen Ende einer Antennularia ramosa wird nicht durch Polarität oder Orientirung des Stücks mit Bezug auf die Schwerkraft bestimmt, auch nicht durch Verhältnisse, die am anderen Ende des Stücks bestehen, es sei denn bei normaler Anheftung des Thieres.

2) Gewisse Stengeltheile haben die Tendenz, Wurzeln hervorzubringen, andere Stämme. Basale Stücke bringen gewöhnlich Stämme hervor; Stücke aus der Mitte erzeugen Wurzeln; Stücke aus der Spitzenregion, aus dem Wachsthumsbezirk geschnitten, haben die Tendenz, den Stamm nach unten fortzusetzen.

3) Ein Stück, dem es nicht gelungen ist, sich mittels seiner Wurzeln anzuheften, oder welches durch wiederholtes Hervorbringen von Wurzeln erschöpft wurde, erzeugt schließlich einen oder mehrere Stämme an den Enden oder seitlich.

4) Das Cönosark von Antennularia ramosa besitzt einen so indifferenten Charakter, dass man es einer Wachsthumsform entziehen und zu einer anderen bringen kann, ohne dass neues Gewebe producirt wird.

5) Die Regeneration bei A. ramosa scheint, wenigstens in den früheren Stadien, mehr die Anpassung des Cönosarks, welches schon gebildet war, an neue Bedingungen und Zwangsverhältnisse darzustellen, als dass dabei unter Zellwucherung der Aufbau neuer Gewebstheile veranlasst wird.

6) Das Cönosark kann von allen Pinnae und Neubildungen hinweg auf den alten Stamm zurückgebracht werden und bleibt dann wochenlang unthätig — wenigstens zwei Monate —, dann treibt es auf einmal schnell neue Wurzeln und Stämme an den Seiten und Enden des Stengels.

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A PRELIMINARY NOTE ON THE ABSORPTION OF THE HYDRANTHS OF HYDROID POLYPS.

H. F. THACHER.

Loeb has found that when pieces of *Campanularia* are placed in dishes of sea water, the polyps in contact with the glass undergo a transformation and disappear completely into the stem. This process, he states, is "due to contact, and is accomplished by the liquefaction and subsequent withdrawal of the protoplasmic mass." In taking up this subject, at the suggestion of Professor Morgan, my wish was to see whether in this case a study of the histology would support Loeb's theory of the liquefaction of the protoplasm as a result of contact: and also whether the process in *Campanularia* resembles that in other hydroids in which absorption occurs, but is due merely to the change from natural to laboratory conditions. For the latter point, in addition to *Campanularia*, I examined *Eudendrium* and a few cases of *Pennaria*, both of which forms also readily absorb their hydranths.

To see if by chance *Campanularia* would also absorb its hydranths when not in contact, I made a set of experiments, placing the splinters of wood on which the hydroids were growing in dishes, so that, as far as possible, the animals would be in a normal position. Under these conditions I found that the polyps were absorbed as rapidly as when touching the glass. These results show at least that contact is not essential to the production of this phenomenon, and suggest the likelihood that the absorption is due to the same cause in all cases.

The beginning of the degenerative changes are first shown by the appearance of large numbers of spherical granules in the digestive current, and by an increase in its rapidity. Shortly after, the polyp, which is to be absorbed, contracts into its cup, and the tentacles fold closely over it. Gradually the polyp becomes shorter and shorter, and the tentacles pass from the length found during ordinary contraction to a knob-like stage, and later are completely absorbed. Towards the close of this period the hypostome also disappears. At this time the digestive current which has been forced periodically from one end to the other of the hydroid-colony, may, by distending the remains of the polyp, delay absorption for a number of hours. If, however, the pressure of the current is not great, the polyp grows gradually smaller until only a small ball of material is left in the cup, and this is then drawn down into the stalk. The whole process may occur in six hours, or may be prolonged for two days or more.

A study of the prepared material shows that changes begin first in the endoderm cells of the body of the polyp, into the cavity of which are thrown fragments of degenerating endoderm and gland cells. This continues for some time, and is accompanied by the contraction of the supporting lamella, as a result of which the ectoderm changes from a flattened to a columnar form. The cells of the hypostome round up rapidly at a comparatively late stage, and are set free into the digestive cavity; the lamella contracting as before. In the tentacles the endoderm is also in process of degeneration, and later, when a break comes in the lamella at the base of the tentacle, the cells pass into the body cavity. The ectoderm cells in this region are thrown into folds which, seen from the surface, might easily give the effect of being fused, as noted by Loeb; but I have not seen any signs of real fusion — only many cases where the cells of different tentacles are brought into close contact. During this time the lamella of the tentacle breaks, and masses of nettle cells and ectoderm pass through the break into the digestive cavity. The broken ends of the lamella now draw together and form a hollow shell, which is frequently much distended by the pressure of the digestive current on the elastic lamella. Degeneration continues by the slow turning in of ectoderm and endoderm cells, until only a small fraction of the original polyp remains, and this is then drawn through the opening at the base of the cup. There are no signs, either external or internal, of any drawing back of protoplasm to form a part of the stalk previous to the final stage, but at this time the strands of protoplasm connecting the cœnosarc and perisarc at the end of the stalk are broken, and in sections the masses of nettle-forming cells, which usually lie in the ectoderm just below the polyp, can be seen to have moved farther down the stalk.

Examination of *Eudendrium* and of *Pennaria* show that the process is the same as that in *Campanularia*, except in those secondary points to which the structural differences of the hydroids would give rise. There being no cup, the tentacles remain separate during absorption, so that there can be no question of fusion taking place. The lower row of tentacles of *Pennaria* persists somewhat longer than do those on the hypostome, but both ultimately disappear, and in neither form is there a withdrawal into the stalk until the polyp has almost entirely degenerated.

The results show that in these three hydroids the method of absorption is the same. No trace of liquefaction of protoplasm, or of withdrawal of the polyp as a whole can be found. The absorption takes place by the degenerating cells of the endoderm and ectoderm being turned into the digestive tract of the colony.

The Gastrulation of the Partial Embryos of Sphaerechinus.

By

T. H. Morgan.

Eingegangen am 29. Oktober 1902.

Owing to the differences in the results obtained by DRIESCH and myself in regard to the number of cells utilized in the development of the partial larvae of the sea-urchin I re-examined the question last year[1]) using Toxopneustes variegatus for the purpose. My results showed that the partial blastulae that gastrulate at the same time as, or very soon after, the normal use a proportionate number of cells in the formation of the archenteron; while those gastrulating later may use a larger number. This result seemed to account for the difference between DRIESCH and myself, since he appears to have observed early gastrulae, while I had later ones. Having an opportunity during the present summer, while holding the Smithsonian Table at the Naples Station, to re-examine this question with Sphaerechinus, I did so, in order to see if in this form also, the one I had used in 1895, the early gastrula uses a proportionate number of cells, and later ones a larger number.

The eggs were fertilized in sea water, and their membranes shaken off immediately. They were then placed in HERBST's calium-free solution, where they remained until segmentation began. At the two- and four-cell stages, eggs were taken out and again shaken until many of the blastomeres were separated. They were then put into sea water, where they continued to develop. The embryos were preserved at intervals, after gastrulation had begun, stained, mounted, and their cells (nuclei) counted.

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[1]) Archiv f. Entwickelungsmech. XIII. 1901.

In the first set some of the whole embryos had just finished gastrulating at 12.30 P. M., while many were not more than half completed. At 1.30 P. M. two $^1/_2$ gastrulae (measuring 15 × 15 divisions[1]) each) had in one case 450 cells in the wall and 53 in the archenteron, and in the other case (the cells in the wall were not counted) 63 cells in the archenteron. At this time a $^1/_4$ gastrula (measuring 11 × 11 divisions) had 227 cells in the wall and about 21 in the archenteron.

At 2.30 P. M. the following $^1/_2$ blastulae and gastrulae were examined (Table I). The whole gastrulae that had completed gastrulation at this time had about 90 to 100 cells in the archenteron.

Table I. 2.30 P. M.
$^1/_2$ Gastrulae.

Size	Stage	Cells in Wall	Cells in Archent.
18 × 18	Blastula	550	
16 × 16	-	425	
17 × 17	-	450	
16 × 16	Begin Gastr.	358 +	
17 × 15	Gastrula not finished	475	33
17 × 15	Gastrula nearly finish.		31

Table II. 2.30 P. M.
$^1/_4$ Gastrulae.

Size	Stage	Cells in Wall	Cells in Archent.
13 × 12	Gastrula not finished	253	12
12 × 12	Gastrula excentric	215	40
13 × 12	Gastrula excentric	250	40

At 3.30 P. M. one $^1/_2$ gastrula (measuring 17 × 16 +) had 38 cells in the archenteron. Two $^1/_4$ gastrulae (measuring 12 × 12 and 11 × 10) had in one case 188 cells in the wall and the beginning of an archenteron (not possible to count the cells), and in the other case 60 cells in the archenteron. In the last case the number appears proportionately too large, although the invagination appeared not to be completed. The archenteron is also proportionately too large.

A 4.30 P. M. a $^1/_2$ blastula (17 × 17) which showed no signs of gastrulating had 580 cells in its wall, another (18 × 18) beginning to gastrulate (20 cells turned in) had 510 cells in the wall. A $^1/_2$ gastrula (not completed) had 470 cells in the wall, and 52 in the archenteron; another (18 × 14) had 468 cells in the wall and 85 in the archenteron; and another (18 × 16) had 55 cells in the archenteron. At this time most of the $^1/_2$ larvae appeared to have about half-gastrulated.

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[1]) In this paper 18 divisions equal $^1/_{10}$ mm.

At the same time (4.30 P. M.) three $^1/_4$ gastrulae gave the following: One (12 × 10), only half-gastrulated, had 195 cells in the wall and 49 in the archenteron. The archenteron was disproportionately broad and large. Another (12 × 11) had 200 cells in the wall and 25 in the archenteron, which had just begun to turn in. A third (12 × 12) had 214 cells in the wall, and had just begun to invaginate.

At 5.30 P. M. the whole gastrulae (26 × 23) had about 125 cells in the archenteron. The two following tables give the results for $^1/_2$ and $^1/_4$ gastrulae at this time.

Table III. 5.30 P. M.
$^1/_2$ Gastrulae.

Size	Stage	Wall	Archent.
19 × 17$^1/_2$	Gastrula	—	50
18$^1/_2$ × 16	-	—	40
18 × 18	Gastrula not finished	—	33
16 × 15	Gastrula	440	88
16 × 14$^1/_2$	-	—	56
16 × 15	-		67
16 × 15	Half Gastr.		60
15 × 15	Gastrula	560	75
19 × 16		475	80

Table IV. 5.30 P. M.
$^1/_4$ Gastrulae.

Size	Stage	Wall	Archent.
14 × 14	Gastr. begun	214	
12 × 12	Blastula	250	
13 × 12$^1/_2$	-	325	
12 × 12	Gastrula excentric	185	25
14 × 12	Gastrula	200	26
13 × 11$^1/_2$	-	250	31
13 × 13	Gastr. begun		23
13 × 12	$^2/_3$ Gastrula		28
12 × 12	$^1/_2$ Gastrula		29
10 × 10	Gastrula	223	28
13 × 11	-	175	48
11 × 9	$^1/_2$ Gastrula	146	33

In Table III it is seen that the number of cells of the archenteron is larger than half of the whole number, although the number in the wall is not more than half. In Table IV a number of $^1/_4$ gastrulae have disproportionately too many archenteric cells, and some of those in which the invagination is not even completed have a few more than a fourth of the whole number.

The results of the second series of observations are given in the following tables, etc.: At 9.30 A. M. the whole blastulae (22 × 22) had about 950 cells in the wall. Two $^1/_2$ blastulae had in the one case (15 × 15) 480 cells in the wall and in the other (14 × 14) 433 cells,

i. e, almost exactly half the whole number. At 11.30 one whole blastula (23 × 23) had about 1000 cells in the wall, and a whole gastrula had about 940 cells in the wall and about 100 in the archenteron. At this time a few whole forms had completed gastrulation. A few of the 1/2 blastulae had begun to invaginate, and I even found three 1/4 blastulae which were also gastrulating. One of these (10 × 10) had 200 cells in the wall and the other (11 1/2 × 11 1/2) 180 cells. One that had about finished gastrulating (11 1/2 × 11 1/2) had 23 cells in the archenteron and about 215 cells in the wall.

At 4.45 P. M. the whole gastrulae swimming at the top had about 1000 cells in the wall, and those that had completed gastrulation had turned in between 90 and 110 cells. Those on the bottom were not quite so far advanced. They had from 800 to 900 cells in the wall, and one that had not completed gastrulation had 50 cells in the archenteron. One 1/2 gastrula (17 × 17) from the top had 304 cells in the wall and about 30 or more in the archenteron. A 1/2 gastrula (19 × 17), from the bottom, had 355 cells in the wall and 40 in the archenteron. A 1/4 gastrula (12 × 8) had 230 cells in the wall, and 16 in the incomplete archenteron; another (13 × 13), from the bottom, had 255 cells in the wall and 25 in the incomplete (?) and very oblique archenteron.

Two hours later, 6.30 P. M., three 1/2 gastrulae from the top (the first three in the table) and two from the bottom gave the following results: —

Table V. 6.30 P. M. 1/2 Gastrulae.

Size	Stage	Wall	Archent.
19 × 19	Gastrula	500	50
20 × 20	-	500	45
20 × 20	-	—	55
19 × 19	-	—	40
19 × 19	-	—	30

At this time the whole gastrulae (28 × 28) had about 100 cells in the archenteron. Two 1/4 gastrulae had, in one case (14 × 14) 260 cells in the wall and 45 in the archenteron, and in the other case (15 × 14) 30 cells in the archenteron.

It will be seen that while the 1/2 gastrulae have about the proportionate number, one, of the 1/4 gastrulae has proportionately too many.

At 9.30 P. M. the whole gastrulae (28×28) had about 125 cells in the archenteron. Three of the $^1/_2$ gastrulae from the top (21×21, 23×20, 20×20) had respectively about 68, 75 and 61 cells in the archenteron. One $^1/_4$ gastrula (15×15) had 240 cells in the wall and 30 in the archenteron, which in this case did not appear disproportionately large, but lay somewhat excentrically. From the bottom the following $^1/_2$ and $^1/_4$ gastrulae were examined.

Table VI. 9.30 P. M.

$^1/_2$ Gastrulae.

Size	Wall	Archent.
20×18	400	60
19×16	415	46 (small)
19×16	400	67

Table VII. 9.30 P. M.

$^1/_4$ Gastrulae.

Size	Wall	Archent.
14×12	240	30
13×13	—	25
15×14	230	35
13×13	240	23
14×13	235	25

General Conclusions.

The principal result is here the same as that which I looked upon as the main one in my first experiments; viz., that there is in the partial larvae no regulation of the cell-size. The cells in the $^1/_2$ larva are proportionately twice too big, as compared with those of the whole larva, and in the $^1/_4$ larva four times too big. The early gastrulae of Sphaerechinus, as in Toxopneustes, turn in, to form the archenteron, a proportionate number of cells. I found again, as I had observed in Toxopneustes, that in a large number of these gastrulae the archenteron is quite excentric. The early whole gastrulae of Sphaerechinus and Toxopneustes are practically radially symmetrical. Hence I can only interpret the excentricity of the archenteron of the partial larva as due to an imcomplete regulation; a trace of the former structure still showing itself in the archenteron of the partial larvae. DRIESCH's criticism[1]) in regard to this point, based on an examination of Echinus, does not touch the problem, for, as the archenteron of the whole larva of this form is in an excentric position there are no means of determining to what the excentricity of the archenteron of the partial larva is due. Moreover the excentricity of the partial larvae of Toxopneustes is much greater than that in Echinus.

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[1]) Archiv f. Entwickelungsmech. XIV. 1902.

I have noticed again, as I had done before both for Sphaerechinus and for Toxopneustes, the disproportionately large size of the archenteron in the $^1/_4$ forms. In part this may be due to the fact that the small blastulae do not enlarge in the same proportion as do the whole, but as Driesch has shown, in proportion to their surfaces, not to their volumes; yet I do not believe this gives a satisfactory explanation of the disparity in size of the archenteron in many of these partial larvae.

It is noticeable, especially in the later gastrulae (see Table IV), that the archenteron, when only half invaginated, may contain as many as, or even more than, one-fourth of the whole number of cells. It was this fact that I described in my first paper, and it was one of the main reasons that led me to conclude that the partial larvae tend to make use of the typical number of cells in forming the archenteron. The explanation that I gave in my last paper is, I believe, more satisfactory; viz., that in the partial blastulae, in which the invagination is retarded, the cells in the archenteric plate slowly increase in number (as do the cells in the archenteron of the whole gatrula after invagination), so that a disproportionately large number may appear even before the gastrulation is completed. One should be very careful to distinguish between these incomplete gastrulae and those that are complete but turned to one side. By rotating the larvae it is easy to tell which of these conditions is present. It is also noticeable in some of the late partial larvae that a proportionately larger area is involved in the inturning of the archenteron, and this is apparently connected with the disproportionate size of the archenteron.

The Number of Cells Invaginated by Strongylocentrotus.

The recent interesting results[1]) obtained by Boveri in regard to the orientation and gastrulation of Strongylocentrotus led him to the conclusion that half of the cells of the blastula-wall is turned in to form the mesenchyme and the archenteron. If this conclusion is correct then Strongylocentrotus differs from the other sea-urchins that I have examined, viz., Sphaerechinus, Echinus, and Toxopneustes, which turn in only about one-tenth of the total number of cells in the blastula. Boveri has possibly overestimated the extent of the inturning of cells in Strongylocentrotus. The question has another bearing of some theoretical interest. If half of the blastula wall becomes endoderm, then Driesch's results from cutting the blastula in two may have a different interpretation, for it would be difficult to obtain a piece of the anterior part of the blastula which does not contain some endodermal cells. If it contained such cells we might not be justified in concluding that every region of the blastula has the power of forming endoderm.

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[1]) Verhandl. phys.-med. Ges. Würzburg. XXXIV. 1901; und Zool. Jahrb. XIV. 1901.

I examined, therefore, with some interest the process of invagination of Strongylocentrotus, and found the same rule holds here that I had made out for the other species. About one-tenth of the whole number of cells is invaginated, and not one-half as Boveri's conclusion demands.

Thus I found about 800 cells in the blastula wall an hour and a half (at 9.30 A. M.) before gastrulation begins. At 11 A. M. gastrulation had just been completed in a few individuals, and in one of these I counted about 800 cells in the wall and about 100 in the archenteron. Again at 4 P. M. there were, in one count, about 900 cells in the wall and 135 in the archenteron. I conclude, therefore, that Boveri's estimate is much too high, for, if it were correct, nearly 400 cells should be turned in, while in reality only about 100, or $^1/_9$ the total number are invaginated. Driesch[1]) has also called attention to this discrepancy, in an article that has just reached me. He gives also the number of cells of the mesenchyme in Strongylocentrotus. He estimates that 50 cells wander in to form this tissue, and if we add this number to the 100 cells of the archenteron, we see that the total number is still far below that of the estimate derived from Boveri's results.

Summary.

1) The $^1/_2$ and $^1/_4$ larvae of Sphaerechinus contain only a half and a fourth respectively of the total number of cells in the whole larva. These cells are, therefore, proportionately twice and four times too large. There is no regulation in cell-size.

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[1]) Archiv f. Entwickelungsmech. XIV. 1902.

2) The $^1/_2$ and $^1/_4$ blastulae that gastrulate at the same time as, or soon after the whole blastulae, use a proportionate number of cells for the archenteron, viz.: one-tenth of the whole number.

3) The archenteron, especially of the early gastrulae, is often very excentric, which is probably due to an incomplete regulation; showing that a trace of the original structure is still present.

4) The partial blastulae that gastrulate later turn in proportionately more cells than one-tenth of the whole number, as in Toxopneustes. When only half-gastrulated they sometimes have more than the proportionate number of cells in the archenteron.

5) A larger area of the archenteric plate is involved in the late partial larvae, and the archenteron, especially in the $^1/_4$ gastrulae, is often disproportionately too large.

6) Strongylocentrotus appears to be governed by the rule followed by other sea-urchins, and invaginates about one-tenth of the whole number of cells, and not one-half as BOVERI's results seem to indicate.

Zusammenfassung.

1) Die ganzen Halbei- und Viertelei-Larven von Sphaerechinus enthalten nur die Hälfte und bezw. ein Viertel der Totalanzahl von Zellen in den Ganzei-Larven. Diese Zellen sind daher, in entsprechendem Verhältnis, zwei- und viermal zu groß. Eine Regulation der Zellgröße giebt es dabei nicht.

2) Die Halbei- und Viertelei-Blastulae, welche gleichzeitig oder bald nach den Ganzei-Blastulae die Gastrula bilden, verwenden eine verhältnismäßig entsprechende Anzahl von Zellen für den Urdarm, nämlich ein Zehntel der Gesammtzahl.

3) Der Urdarm ist oft, ganz besonders in den frühzeitig gebildeten Gastrulae, sehr excentrisch, was wahrscheinlich auf einer unvollständigen Regulation beruht; es zeigt dies, dass noch eine Erinnerung an die eigentlich normalen Bauverhältnisse besteht.

4) Die später gastrulirenden Eitheil-Gastrulae stülpen verhältnismäßig mehr Zellen ein, als ein Zehntel der Gesammtzahl, wie bei Toxopneustes. Wenn die Gastrulation erst halb vollendet ist, haben sie manchmal mehr als die verhältnismäßige Zellenzahl im Urdarm.

5) Ein noch größeres Feld der Urdarmplatte wird in den späten Theillarven eingestülpt, und der Urdarm ist oft, speciell bei den Viertelei-Gastrulae, zu groß.

6) Für Strongylocentrotus scheinen dieselben Regeln zu gelten, denen die anderen Seeigelarten folgen. Er stülpt etwa ein Zehntel der gesammten Zellenzahl ein, und nicht die Hälfte, wie BOVERI's Ergebnisse anzudeuten scheinen.

Some Factors in the Regeneration of Tubularia.

By

T. H. Morgan.

With 16 figures in text.

Eingegangen am 29. Oktober 1902.

The following experiments were carried out at the Naples Stazione Zoologica between June 15, and Aug. 15, 1902, while occupying the table of the Smithsonian Institution. I desire to express my obligation to Prof. LANGLEY and to the committee of the Smithsonian Table for granting me once more the opportunity of working at Naples.

Influence of the Size of the Piece and of the Region of the Stem from which it Comes.

DRIESCH was the first to show that the incomplete structures that BICKFORD and he himself, in particular, had found when very short pieces of the stem are cut off, appear more often in pieces from the distal region of the stem. Later I obtained the same result¹), and noticed further that much longer pieces from the distal region produce incomplete structures than from the more proximal regions. I tried to show that this peculiarity is also associated with individual stems, in some of which the region forming incomplete structures is more extensive than in others, and I suggested that this difference might be due to the age of the stem — the distal region of younger stems having a stronger tendency to produce incomplete structures than the same region in older stems. In a later paper²) I pointed out that the relative thinness of the living part — the coenosarc — near

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¹) Archiv f. Entwickelungsmech. XI. 1901.

² Archiv f. Entwickelungsmech. XIII. 1902.

the distal end, as compared with the more proximal regions, might account for the fact that longer pieces from the distal region give incomplete structures[1]. There remained a number of points in this experiment that needed further examination. First, it was desirable to obtain more cases to establish the view that different stems behave differently. Second, longer series were needed extending further into the proximal region. Third, I hoped to be able to make out why sometimes single incomplete structures develop, and at other times double structures; and also the relation between these two kinds of structures.

The following six series include eight short pieces each. The first piece in each is the distal one. The same abbreviations are used that were employed in my former papers; thus hy. = hydranth; pb. = proboscis; hy. + stk. = hydranth plus short stalk; hy. + stem = hydranth plus stem; i. e., proximal coenosarc in contact with perisarc. The double structures are indicated by the letter d; thus, d.pb. = double proboscis. Throughout this paper 30 divisions are equivalent to one millimeter.

No. I.		No. II.		No. III.	
35	hy. + short tent.	35	pb.	48	no structure
34	d.pb.	30	d.pb.	50	d.pb.
15	dead	28	d.pb.	38	d.pb.
35	pb. + short tent.	34	hy. + stk.	41	hy. + stem
37	d.pb.	36	hy. + stem	44	- -
32	d.pb.	38	- -	39	- -
31	d.pb.	34	- -	40	- -
34	d.pb.	27	- -	40	- -

No. IV.		No. V.		No. VI.	
31	dead	38	pb.	48	dead
27	hy.	44	hy. + rud. tent.	48	hy. + repr. + 2 tent.
32	d.pb.	42	d.pb.	42	d.pb.
35	hy.	48	d.pb.	48	d.pb. + repro.
30	d.pb.	32	hy. + stem	55	- -
38	-	52	- -	52	hydranth
30	-	38	- -	45	d.hy. + one circ. tent.
40	-	45	- -	50	- - - -

The following series is from another set that developed very slowly, yet appeared otherwise in good condition. The stems were noticeably exceptionally long, and the colonies may have lived under peculiar conditions. The slowness of development was not due to cooler weather, or to any other condition of the experiment that I could detect. This series contained 19 consecutive pieces.

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[1] Whether the thinness is associated with the age of the region I do not known, but it seems probable that it is.

No. VII.

1)	47	nothing
2)	49	dead
3)	50	d.pb.
4)	52	d.pb.
5)	56	hy. + stk.
6)	50	d.hy.
7)	62	d.pb. + 2 repro.
8)	70	hy. + stem
9)	56	d.pb.
10)	60	hy. + stem
11)	49	d.pb. + repro.
12)	70	d.hy.
13)	30	hy. + stem
14)	85	hy. + stem
15)	55	d.pb. + repro.
16)	80	hy. + stem
17)	50	d.pb. + repro.
18)	30	d.hy.
19)	42	d.pb. + repro.

If we examine the preceding seven cases we find striking differences, but as I pointed out on another occasion, it is unprofitable, owing to the occurrence of double structures, to compare the series with one another. If we knew the relation between these two kinds of structures, the double and the single, the comparison might still be possible, but we do not understand this relation. In the first and fourth table there appears to be a marked tendency to produce double proboscides as compared with tables II, III and V. In the sixth table a single hydranth appeared in the sixth piece, while the two following pieces, although fairly long, made double hydranths with one circle of tentacles, which is unusual except near the distal end. This might be interpreted to mean that in this piece the region forming incomplete structures extends further back than usual. On the whole the results were in harmony with my previous ones on T. crocea, but were still insufficient to establish my point of view. I tried, therefore, in other ways to get some light on the subject. In the first place, in a number of cases, alternately long and short pieces were cut off in order to discover if possible, what relation the single and double structures bear to each other. The following series give some of the results.

These three tables, selected from a series of ten, give very little further information on the obscure problem of the relation between single and double structures. Certain points can, however, be made out. First, a short piece from any region of the stem may make a double proboscis. Moreover, we can not assume that this double structure corresponds to any particular incomplete structure. In fact, the third series shows that it is present in regions in which somewhat longer pieces made a hydranth and stem. It is found that the

No. VIII.		No. IX.		No. X.	
41	dead	65	dead	70	pb. + repro.
20	-	41	-	43	d.pb.
38	-	75	hy. + stem	65	hy. + stem
28	-	31	dead	38	d.pb.
61	hy. + stk.	51	hy. + stem	62	hy. + stem
30	pb. + repro.	16	dead	35	d.pb.
50	hy. + stem	42	pb. w. 1 tent.	60	hy. + stem
25	d.pb.	75	d.hy. 1 circ. tent.	35	d.pb.
41	d.pb. + repro.	32	d.pb.	52	hy. + stem
36	hydranth	75	d.hy. + 2 circ. tent.	35	d.pb.
40	hy. + stem	36	d.pb.	60	hy. + stem
25	d.pb. + repro.	80	dead	31	d.pb.
51	hy. + stem	30	-	50	hy. + stem
59	- -	73	d.pb. + 3 tent.	24	dead
50	d.pb. w. 2 tent.	28	d.pb.		
27	hy. + few tent.				

double proboscis occurs in pieces that are less in length than that of the anlage of the normal hydranth (about 50 divisions). **Driesch** has shown that in short pieces the length of the hydranth-forming region is reduced. If we assume that the influence (producing an aboral hydranth) from the cut aboral end is stronger than the reducing power of the piece, then it may gain the ascendency, and a double structure be the result. This seems to me to be at least a plausible view. Conversely, in pieces sufficiently long for the oral hydranth alone to develop (it may be more or less reduced) the formation of this hydranth counteracts, for a time at least, the influences at the cut aboral end that tend to produce an aboral hydranth. It is quite certain in a large number of cases that the double proboscis, or double hydranth, develops before the aboral head appears on other short pieces with a hydranth at the oral end.

If this view is probable for the double proboscis, may we not extend it to other double forms which arise in pieces as long as or even longer than the normal hydranth-forming region; for, as **Driesch**'s results have shown, the reduction of the hydranth-forming region occurs in pieces that are much longer than the hydranth-forming region itself. In this case also the influence from the aboral end is stronger than the reducing influence, and a double hydranth appears, often with a single circle of tentacles between the two ends. The method of development of such a piece shows that proximal tentacles are produced on both sides, but since they arise with their bases near together, the hydranth appears when freed from its tube to have only a single circle common to the two ends. It is very rare to find two complete hydranths, one on each end, with only a short connecting region, and this result fits in with the provisional hypothesis that I have given.

In a former paper (1902) I have shown that if a piece of the stem is tightly constricted at one region, by tying a thread around it, and if then the stem is cut off close to the constricted part, the small piece that is left may produce an incomplete structure with the distal end of the new part always towards the free cut-end regardless of whether this is the oral, or the aboral end of the original piece. This result demonstrates that the influence producing the double structures arises from the open ends of the piece, without respect as to whether the open end is the oral, or aboral end. This conclusion also fits in well with my provisional hypothesis. I repeated during the past summer this same experiment on the Neapolitan form, and obtained the same results that I had obtained previously on T. crocea.

We might obtain by means of this experiment a series of single structures all from the same stem, and in this way we might hope to determine more satisfactorily whether, as I suppose, individual stems have a greater or less tendency to make incomplete structures; but the technical difficulties of tying the knots so near together was sufficiently great to discourage me from making the attempt. Another method suggested itself. If the short pieces could be made to stand up on one end in sand, then single structures I thought might appear only at the free end. In the next section the results of this experiment are given.

Short Pieces Standing on End in Sand[1]).

Consecutive pieces were cut from the stem and planted in a row in fine sand, so that one end was buried and the other was above the surface of the sand and surrounded by the water. In one series all the oral ends of the pieces were up, in another the aboral ends. Owing to the difficulty of keeping such small pieces properly orientated after cutting, it was found necessary to cut them off alternately obliquely and squarely, so that each piece had one square and one oblique end, which in consecutive pieces were alternately uppermost. A series of check experiments showed that this slight obliquity of the end does not interfere with the formation of partial structures, etc.

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[1]) It would be better, I think, to use perforated plates of aluminium, and stand the pieces in the holes, one end touching the bottom of the dish.

The length of the pieces is that of the longest side of the perisarc, and since some of the pieces were quite oblique the measurements make them appear longer than they really were. They were, it is true, often longer than those in the first series of tables (because very short pieces were difficult to keep upright in the sand), yet most of the pieces were short enough to have produced incomplete structures if they had lain flat on the bottom of a dish, and a few series were cut specially short.

No. XI.

63	pb.
58	hy. + stem
65	- -
48	?
40	hy. + stem

No. XII.

58	hy. + stem
50	- -
45	- -
40	- -
40	- -
45	- -

No. XIII.

52	pb. + repro. rud.
45	- - -
41	- - -
47	hy. + short stk.
37	hy. + stem
52	- -
34	- -
40	- -

No. XIV.

45	pb.
30	pb. + few tent.
45	hy. + stk.
40	hy. + stk.
39	hy. + stem
38	hy. + stem

No. XIV A.

35	hy. + stk.
48	- -
36	- -
60	hy. + stem
45	- -
50	- -

No. XV.

40	hy. + stk.
53	hy. + stem
45	- -
50	- -
32	- -

No. XVI.

45	d.pb.
—	pb. + repro.
55	hy. + stem
60	- -
65	- -
60	- -

In the four following series the aboral ends were up.

No. XVII.

35	pb.
39	pb.
60	hy. + stk.
—	- -
45	pb.

No. XVIII.

50	hy. + stem
40	- -
45	- -
60	- -
50	- -
49	- -

No. XIX.

80	hy. + few tent.
—	hy.+shorttent.
—	hydranth
58	hy. + stk.
57	hy. + stk.

No. XX.

75	hy. + stem
65	- -
60	- -
60	- -

In the following series very short pieces were cut off (as a rule squarely) and put into the sand without regard to which end of each piece was uppermost. It was very difficult to keep them all standing up. Those that were found later out on the surface lying on their sides have an (s) placed after them in the tables.

No. XXI.

35	(s)	pb.
30	(s)	-
28		hy. + stem
28		hy. + stk.
30		hy. + stem
27		not develop.

No. XXII.

27	pb. + repro.?
30	pb. + repro.
30	hy. + stem
27	not develop.
37	- -
29	- -

No. XXIII.

35		hy. + repro.
37		- -
25	(s)	d.pb.
32		hy. + stem
25		- -

No. XXIV.

32	hy. + short stk.
35	hydranth
36	hy. + stem
21	- -
31	- -
23	- -

No. XXV.

30	pb. + repro.
32	hy. + stk.
28	pb. + repro.
25	hydranth
25	hy. + stem

No. XXVI.

15	dead
20	-
20	pb.
28	hy. + stem.
28	- -

The tables make it quite clear that by placing pieces in the sand only single structures develop, either incomplete or whole, but more often the latter. Moreover, this occurs also when the aboral end of the piece is uppermost. In only two cases did a double proboscis appear (when the piece remained in the sand), but if we consider that the sand was rather coarse, the lower end of the piece may have been free in an opening between the particles of sand. These two cases out of several hundred do not affect the general result.

Another point is almost equally clear; viz., there are produced relatively fewer incomplete structures than when similar pieces lie on their sides. It is difficult to show conclusively that this is the case, but in looking over the pieces I was continually impressed by the tendency shown in the smallest pieces to produce a stalk or a stem in the buried end. Another factor appears, therefore, to have been introduced, so that it is dangerous to compare these results with those of free pieces. Yet, on the whole, the different stems showed individual differences.

The development of these pieces was generally slower than that of similar pieces lying on the bottom of a glass dish. The delay may be due to lack of oxygen, or to the presence of carbon dioxide near the surface of the sand, but I have made no special experiments to test these possibilities.

The Number of Tentacles in Pieces with Reduced Tentacle-Anlagen.

In a previous paper I pointed out that in very small pieces, in which the tentacle-anlage is very much reduced, the number of tentacles is also smaller. I have examined this question more thoroughly and find that such an unconditioned statement is not correct. The most important fact in this connection is that the anlage of the tentacles may be very much shortened lengthwise without a corresponding reduction in the number of tentacles; also, the diameter of the piece is the most important factor in determining the number of the new tentacles. It is not improbable that the thickness of the coenosarc, as well as the region of the stem (with which the thickness of the coenosarc may be connected) may also enter into the result.

Very short and very long pieces of the same stem were cut off alternately and the proximal tentacles counted as soon as the hydranth emerged[1]. The following tables give some of the results. Sometimes two or more consecutive short pieces were cut off. In such cases they are bracketed. The diameter of the stems is also given in most cases.

No. XXVII.

⎧	20	no development
⎩	25	- -
	200	- -
	25	d.pb.
	240 × 11.	13 tent.
⎧	28 × 10½.	12 (or 13) tent.
⎩	25	hydranth
	210	12 tent. (2 double)
⎧	36 × 10.	10 tent.
⎪	20	d.pb.
⎪	20	-
⎩	18	-
	350	dist. × 10.12 tent. prox. × 8.10 tent.

No. XXVIII.

⎧	35	d.pb.
⎩	45	hydranth
	200 × 13.	12 tent.
⎧	24	died
⎩	27	-
	150 × 12.	12 tent.
⎧	28	d.pb.
⎩	15	died
	200	12 tent.
	18	d.pb.
	260	dist. × 14.13 tent. prox. × 9. 9 tent. 1 forked

No. XXIX.

⎧	48	
⎨	52	
⎩	34 × 13 .	12 tent.
	230 × 13 .	14 tent.
⎧	30	12 tent. (one double)
⎩	38	15 tent.
	170 × 10 .	11 tent.
⎧	35	13 tent.
⎩	30	14 tent.
	150 × 11 .	12 tent.
	170 × 11 .	12 tent.

No. XXX.

	40	pb. + rud. tent.
	250 × 12.	14 tent.
	50	dead
	180	14 tent.

⎧	30	17 tent.
⎨	30	
⎩	42	

	300	15 tent.
⎧	20	10 tent.
⎩	30	16 tent.
	285	14 tent.

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[1] The number of tentacles may increase after the hydranth has been formed.

No. XXXI.

65 × 15	12 tent.
Long. $^3/_4$ md.	prox. 16 tent. dist. 16 tent.
36	12 tent.
260 × 15	13 -
42 × 14	18 -
150 × 14	13 -
30	dead
300 × 13	16 tent.

No. XXXII.

85 × 14	18 tent.
350	16 -
80	14 -
85	abnormal
350	16 tent.

No. XXXIII.

80 × 14	d.pb.
250	16 tent.
45	12 -
28	—
40	12 tent.
35	—

No. XXXIV.

50 × 10	d.pb.
185	12 tent.
36	13 -
250	13 -
27	10 -

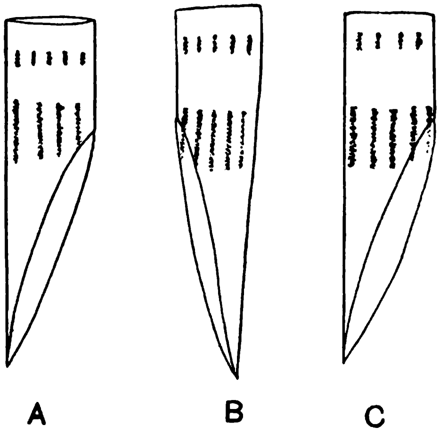
No. XXXV.

95 × 16	d.pb + repro.	50	14 tent.
250	16 tent.	400 × 10 .	10 -
48	d.pb.	38	12 -
250	15 tent.	300 × 9 .	10 -
65	15 -	50	7 -
340	14 -	550	12 -
105 × 14	prox.? 16 dist.? 12		
300	14 tent.		

These tables bring out certain points, but they also show such striking differences that they must be used with caution. In the first place it is seen that pieces with larger diameters produce more tentacles than do those with smaller. The distal region of the stem is larger than the more proximal, and more tentacles are produced by pieces from this region, probably because it is larger, but there may also be other factors. Sometimes the shorter pieces appear to produce fewer tentacles, but in other cases they have the same number as the larger ones, or rarely even more, as seen in No. XXX and XXXI. There is a certain range of variation in the number of tentacles which makes it difficult to compare long and short pieces, but one result is perfectly clear, and it is the all-important one; viz., that the tentacle-anlagen may be reduced a half, or even a fourth or less in length without a corresponding reduction in the number of the tentacles. This result is important in connection with the whole question of proportionate development, or of form-regulation, since it shows that the structural basis may be changed in one direction without being affected in another. Moreover, I think we can give an explanation of this result in the case of Tubularia. The coenosarc is held in contact with the perisarc, so that even in a small piece the diameter remains the same, and on this depends the number of tentacles that are laid down[1]. In other forms, such as hydra, the piece may begin to change its form before the tentacles develop, so that a smaller number are formed in a smaller piece than in a larger one. Whatever the nature of the factors may be, vitalistic or physical, the preceding results with Tubularia seem to show that a physical factor in the piece enters into the result of proportionate development as a determining influence. Those who hold to a vitalistic conception will be obliged to admit that the vitalistic principle may be directly and largely controlled by such a very simple physical factor as keeping a piece stretched to its full size.

The Influence of an Oblique Proximal End on the Formation of the Distal Hydranth in Small Pieces.

I carried out this same experiment once before (1901) on T. crocea, but since I had only a few examples I wished to test again the provisional conclusion that I then reached. It appeared that an oblique basal cut-surface does not determine a reduced, oblique tentacle-anlage, if the latter forms a distal hydranth (and not a proximal heteromorphic hydranth). Pieces were cut off as shown in Figs. *A—E*. In *A*, although the tentacle-anlage is much reduced (about one-third normal length), the proximal row of tentacles is not oblique at either end. In *B* the anlage extends into the obliquely-cut region, but the distal ends of the proximal tentacles lie squarely across the tube. The proximal ends of the same tentacles are, at first, not so well developed where they extend into the oblique region. This, however, is very different from the obliquity of the tentacle-anlage when the



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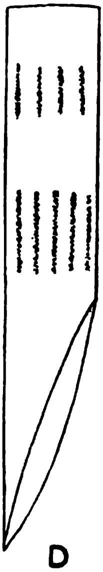
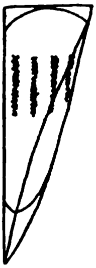
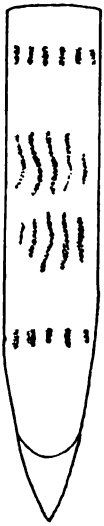
[1] In those cases in which the coenosarc draws away from the perisarc the number of tentacles may be reduced; as shown in the case I observed and figured in my paper in 1901 Fig. *Z*.

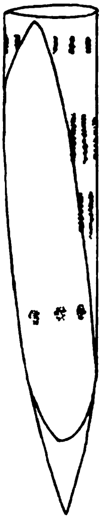
distal end of a piece is cut obliquely. The same lack of development over a newly closed-in region may be observed when a piece is split longitudinally.

Similar conditions to those seen in the last figure are also present in Figs. *C* and *E*. In Fig. *D* (a longer piece) the result is like that in Fig. *A*. In this last case the anlage is less shortened and does not extend so near the oblique cut. When double structures develop, so that an oral structure is formed at each cut-end of the piece, the anlagen of the proximal end may be obliquely placed, but this, of course, is not different from the same phenomenon at the distal end.


D


E

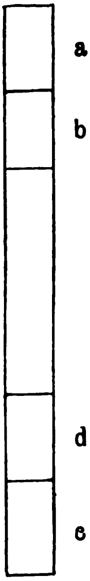

F


F′

The results show that the influences determining the position of the tentacle-anlage emanate from the free end of the piece (which may be either proximal or distal), and the position of the anlage is not influenced from an aboral direction, except in so far as the development of the tentacles may be slower when they extend into a region that has recently closed over. This slowness of the development over a new area is well shown in Fig. *F—F′* in which a double hydranth-anlage is forming. On the old coenosarcal side the tentacle-ridges are well advanced, while on the new side they are scarcely seen. I have also observed that a smaller number of tentacles is formed, if the region of the anlage extends into the oblique part of the piece; and this result confirms the conclusion I reached in the last section in regard to the factors determining the number of tentacles; for the diameter is less in the oblique part.

Single Structures Produced by Allowing One End to Close before the Other.

If a long piece of the stem is cut off and its two ends allowed to close, and if then after one, two, three, or more hours a very short piece is cut off from each end, will single structures in preference to double ones develop from such pieces? Since the relation of the initial polarity is different at the two ends we should expect that the small piece from the distal end of the long piece might behave differently from the one from the proximal end.



G

If single structures (whether whole or incomplete) were formed in this experiment, there are two possible interpretations that might account for them. Either the advantage which one end has been given by closing before the other may be the determining factor in the result, or the early development of the anlage of the hydranth may be the main factor. It is also conceivable that at an early stage one of these factors might determine the result, and at a later stage the other. These alternatives will be discussed after the evidence has been given.

The head of the old hydranth was cut off several millimeters from its attachment. The stem was then cut off two or three (or even more) centimeters from the first cut[1]. The two cut-ends closed in in the course of half-an-hour. After one, two, three, or more hours (from the time of the first cutting) a small piece (*a*) of the distal end (Fig. *G*) was cut off. At the same time a similar piece (*c*) was cut off from the proximal end. Two other pieces, one distal (*b*), one proximal (*d*), were also immediately cut off. These differed from the former two in being open at both ends. The following tables give the main results. In some of the later experiments one or the other end of the small pieces was cut off slightly obliquely in order to examine the orientation of the new structure. The »time« is that between the first and the second cutting.

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[1] The cutting here, as everywhere else in these experiments was, of course, always done under the water.

Time	Length	Result
3 hrs.		
a	49	hy. + short stk.
b	51	hy. + stem
c	37	- -
d	?	—
a	44	hy. + stem
b	46	- -
c	35	- -
d	55	- -
6 hrs.		
a	35	hydranth
b	35	dead
c	28	dead
d	35	hy. + stk.
a	35	hy. + repro.
b	37	d.hy. + tent.
c	—	dead
d	30	d.pb. (?)

Time	Length	Result
6 hrs.		
a	28	pb.
b	42	hy. + short stk.
c	40	pb. + repro.
d	30	nothing
a	50	dead
b	50	hy. + stem
c	40	hydranth
d	38	d.pb.
12 hrs.		
a	32	pb.
b	38	pb. + repro.
c	22	d.pb.
d	too small	

In the following cases a number of stems were cut off at the same time, and all the »*a*«-pieces kept together; also the *b*, *c*, and *d*-pieces.

Time	Length	Result
7 hrs.		
a	48	hydranth
a	40	pb. + repro.
a	42	hydranth
a	—	-
a	42	-
a	37	pb. + repro.
a	40	- -
a	—	- -
a	—	hy. + stk.
b	60	hy. + stk.
b		- -
b		hydranth
b		-

Time	Length	Result
4 hrs.		
a	22	hy. + rud. tent.
a	33	- - - -
a	42	hy. + short stk.
b	25	d.pb.
b	50	-
c		all dead
d	—	d.pb.
d	—	-
d	—	hydranth
d	—	-

Time	Result
5 hrs.	
a	hydranth
a	-
a	-
a	hy. + stk.
b	d.pb.
b	hy. + stem
b	- -
b	hy. + stk.
b	hydranth

Time	Length	Result
2 hrs.		
a	40	hy. + stem
a	40	hydranth
a	45	hy. + stem
a	35	- -
a	40	hydranth
a	—	-
a	20	hy. + stem
a	—	hydranth
b	45	d.pb.
b	40	hy. + stem
b	45	- -
b	45	hydranth
b	47	d.hyd.
b	30	hy. + stk.
c	32	hydranth
c	35	hy. + stk.
c	35	d.hy.
c	25	d.pb.
c	32	-
d	35	d.pb.
d	32	-
d	31	hydranth
d	31	-
d	30	pb. + repro.

Time	Length	Result
1 hr.		
a	60	hy. + stk.
a	50	- -
a	55	- -
a	40	- -
a	50	hydranth
a	70	pb. + repro.
b	—	hy. + stem
b	—	- -
b	—	- -
b	—	- -
b	—	- -
c	50	hy. + stem
c	60	- -
c	36	- -
c	—	d.hy. + stem

Time	Length	Result
3 hrs.		
a	48	hy. + stk.
a	28	hydranth
a	40	hy. + red. tent.
a	42	d.pb.
b	40	pb. + repro.
b	—	hydranth
b	38	-
b	40	hy. + stem
b	30	hy. + stk.
c	22	nothing
c	30	hy. + stem
c	32	- -
c	32	hy. + stk.
d	25	hy. + stk.
d	75	hydranth
d	35	-

Time	Length	Result
2 hrs.		
a	50	d.pb.
a	60	hydranth
a	40	d.pb.
a	40	-
a	—	hy. + stem
a	—	dead
b	55	d.hy.
b	35	d.pb.
b	45	-
b	52	-
c	60	hy. + stem
c	60	- -
c	50	- -
d	60	hy. + stem
d	60	- -
d	65	hy. + repro.
d	50	hy. + stem

Time	Length	Result
3 hrs.		
a	55	d.pb. + repro.
a	45	hy. + stem
a	40	d.pb.
b	50	hy. + stem
b	30	d.pb.
b	—	-
b	—	pb. + 6 tent.
b	—	hy. + stem
c	—	not develop.
c	—	- -
c	—	hy. + stem
c	—	- -
d	—	hy. + stem
d	—	- -

Time	Length	Result
2 hrs.		
a	39	hydranth
a	40	-
a	42	d.hy. + rud. tent.
b	30	d.pb.
b	40	d.pb. + repro.
b	41	hy. + stem
c	45	d.hy. + stem
c	42	- -
c	50	hy. + stem
c	30	- -
c	28	- -
d	—	hy. + stem
d	—	- -
d	—	- -

Time	Length	Result
1 hr.		
a	30	pb.
a	30	-
a	35	pb. + repro.
—	—	3 dead, 3 nothing
b	35	d.pb.
b	29	-
b	—	pb. + repro.
b	28	hy. + stem
b	40	hy. + stk.
b	—	- -
c	35	hy. + stk.
c	39	hy. + stem
c	—	pb.
c	35	d.hy.
c	—	hy. + stk.
d	—	hy. + stem
d	—	d.pb.

Time	Length	Result
3 hrs.		
a	30	pb. + repro.
a	32	- -
a	32	d.pb.
b	40	pb. + rud. tent.
b	39	d.pb.
c	42	hy. + stem
c	39	- -
c	52	d.pb. + one circ. tent.
d	38	hy. + stem
d	35	- -

The main conclusion which we may draw from the preceding results is that if a sufficiently long time elapses between the two cuttings the distal end piece, *a*, generally gives single structures. It is difficult and even impossible to determine the exact limit of time, since this varies in different individuals, and other factors also complicate the result.

The second pieces, *b*, give more often double structures. It may appear that this proves that the results with the *a*-pieces are due to the earlier closure of one end rather than to the laying down of the anlage of the hydranth, but we should not be entirely justified in drawing this conclusion, since we are comparing different regions of the stem. It is possible that the differentiation might be more advanced at the distal end than behind this region. Nevertheless the results appear to indicate that the production of single structures, under the conditions of the experiment, is due to the advantage that the closed distal end has over the open proximal end.

In regard to the end-pieces, *c*, from the proximal end of the long stem, we can see that there are two opposite tendencies at work; viz., 1) the polarity, and 2) the influences at the free proximal end. These pieces give a noticeably large number of single structures, but more double structures than do the *a*-pieces. Since I did not attempt, in most cases, to determine the orientation of the single structures, I do not know which of the two influences, acting on the pieces, predominates.

In the *d*-pieces also there will be the influence of polarity, opposed to which may be the original influence from the proximal end of the original long piece. The results show that double structures frequently appeared.

On the whole it appears that the problem is too complicated to be solved in this way. The single result that stands out clearly, and the one for which the experiment was primarily carried out, is the large number of single structures that are produced by the distal piece, *a*, and this is the result of allowing one end of the piece to close before the other end. Since many of these single structures were hydranths with a stem and not incomplete structures, it can not be claimed that the results are due to the cutting off of distal pieces of the hydranth-forming region. This is true because the hydranths on the small »*a*«-pieces were greatly reduced in size in comparison with the ones that would have developed at the distal end had the pieces been left intact.

The Influence of the Earlier Closing of One End of Long Pieces.

The questions connected with the preponderating influence of the earlier closure of one end suggested the following experiments with longer pieces. A long piece (2—5 cm) was cut off[1], and the ends *A* and *b* (Fig. *H*) allowed to close. Then after an interval of one, two, three, or more hours the piece was cut in two in the middle (at *a*—*B*), so that two new cut-ends were exposed, one, *a*, at the proximal end of the piece *A*—*a*, and the other, *b*, at the distal end of piece *B*—*b*. Other check-pieces were not cut in two.

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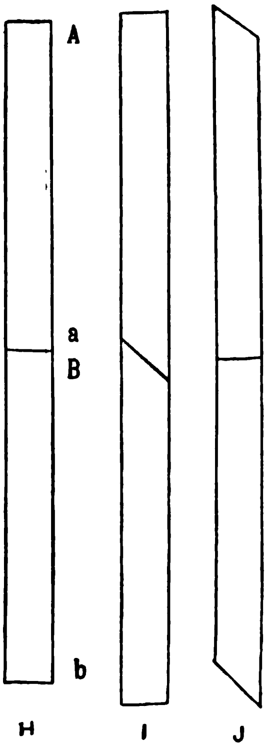
[1] The distal end is in all cases a few millimeters behind the old head.

The problem is to find out if the development of the aboral hydranth at *b* in the piece *B*—*b* is hastened in comparison with the development of the aboral hydranth in check-pieces not cut in two in the middle. It will be observed that the closed end, *b*, has one or more hours start on the oral end, *B*, of the same piece. In the first tables the pieces had all ends cut squarely across (Fig. *H*); the first column gives the time between the first and second cuts; the other columns give the time in hours at which the later observations were made, reckoned from the time of the first cutting.



After 2 hrs.	$26^{1}/_{2}$	$30^{1}/_{2}$	32
A	oral	oral	oral
a	0	0	0
B	0	2 oral 2 aboral 1 both	2 oral 2 aboral 4 both
b	0		

After 3 hrs.	$26^{1}/_{2}$	$30^{1}/_{2}$	32
A	oral	oral	oral
a	0	0	0
B	0	1 oral 4 aboral	3 oral 3 aboral 4 both
b	0		

After 5 hrs.	$26^{1}/_{2}$	$30^{1}/_{2}$	32
A	oral	oral	oral
a	0	0	0
B *b*	1 aboral	2 oral 4 aboral	1 oral 3 aboral 4 both

Conclusions. In this experiment the results seemed clearly to show a hastening of the aboral (proximal) development of the piece

B—b in consequence of its separation from the anterior part, and even the two hours start given to the proximal end seemed sufficient[1], in a number of cases, to bring about the result. In several cases both ends of *B—b* produced heads simultaneously, but this also meant a hastening of the aboral development. Other and later

After 1 hr.	21	22	26	27½	48
A *a*	2 oral	2 oral	4 oral	5 oral	5 oral 6 both
B *b*		3 oral	4 oral	4 oral	5 oral
After 2 hrs.					
A *a*	2 oral	6 oral	6 oral	6 oral	5 oral 1 both
B *b*			5 oral	6 oral	6 oral
After 3 hrs.					
A *a*	3 oral	3 oral	5 oral	6 oral	
B *b*			2 oral	2 oral 1 aboral 1 nothing	3 oral 1 both 1 aboral

After 2 hrs.	21	23½	26½	31½
A *a*	none	2 oral	2 oral (rest poor)	2 oral
B *b*	1 oral (begins)	6 oral	6 oral	5 oral 1 both
After 3 hrs.				
A *a*	3 oral (begin)	5 oral	5 oral ?	
B *b*		2 oral		3 oral 2 both
After 4 hrs.				
A *a*	3 oral (begin)	4 oral	4 oral	
B *b*			2 oral 1 aboral	1 oral 1 aboral 1 both

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[1] At 32 hours in the first table only one of the check, uncut pieces had both an oral and an aboral head; and in the third table also at 30 hours only one check had both.

experiments did not give such striking results, as the following tables show. In the first of the two tables (page 142), the first cut was oblique and the second square, Fig. *J*; in the second, the first cut was square and the second oblique, Fig. *I*. (See page 141.)

In the first of these last two tables there is no influence shown, as a result of the second cutting, except after 3 hours. It should be noted that the end, *b*, was oblique. In the second there appears to be a slight influence observable. In this case the end, *b*, was square.

After 2 hrs.	21	$23^{1}/_{2}$	$26^{1}/_{2}$	$31^{1}/_{2}$
A *a*	1 oral	4 oral	6 oral	5 oral 1 both
B *b*	1 oral	4 oral 1 aboral	4 oral 1 aboral	5 oral 1 both
After 3 hrs.				
A *a*	4 oral	5 oral	5 oral	5 oral
B *b*	2 oral	5 oral	5 oral	5 oral
After 4 hrs.				
A *a*	1 oral	3 oral	4 oral	4 oral
B *b*			3 oral 1 aboral	3 oral 1 both

After 4 hrs.	$23^{1}/_{2}$	$26^{1}/_{2}$	$30^{1}/_{2}$	48
A *a*	5 oral	7 oral	7 oral	7 oral
B *b*	1 oral	2 oral	4 oral 1 aboral 2 none	1 oral 3 both
After 6 hrs.				
A *a*	poor	—	—	1 both rest dead
B *b*	1 oral	1 oral	5 oral 1 both	5 oral 1 both
After 7 hrs.				
A *a*	5 oral	5 oral	5 oral	5 oral
B *b*	2 oral 1 both	1 oral 3 both	2 oral 3 both	2 oral 3 both

In the first of the preceding tables the *b*-end was oblique, and little influence of the second cutting is observable, while in the second table, the interval being longer, however, the influence is more obvious; and in this set the *b*-end was square. In the next two tables the results of cutting long and short ($^1/_2$ inch) pieces in two is shown. In both cases the first cut was square and the second oblique.

After 1 hr.	48	55	57	72
A *a*	6 oral	6 oral	6 oral	6 oral
B *b*		1 oral	3 oral 1 aboral 1 both	1 oral 2 aboral 2 both
After 2 hrs.				
A *a*	6 oral	6 oral	6 oral	6 oral
B *b*		1 aboral	2 aboral	1 oral 2 aboral 1 both
After 3 hrs.				
A *a*	5 oral	5 oral	5 oral	6 oral
B *b*		2 aboral	3 aboral	1 oral 5 aboral

After 1 hr.	24	30	4
A *a*	7 oral	7 oral	7 o
B *b*	6 oral	7 oral 1 both	
After 2 hrs.			
A *a*	8 oral	8 oral	8 o
B *b*	2 oral 1 aboral 1 both	2 oral 1 aboral 1 both	4 o 1 a 1 b
After 3 hrs.			
A *a*	7 oral	7 oral	7 o
B *b*	2 oral 2 aboral	3 oral 2 aboral	4 o 3 a

In the last two tables the influence of the cutting is obvious, and it should be noticed that here, as in the first two tables of this experiment, the interval between the two operations is short, and that the *b*-end is square. In the next two tables a long interval, 8 hours was chosen. In the first of these tables the *b*-end was oblique; in the second, square.

After 8 hrs.	25	$30^1/_2$	38	48
A *a*	18 oral	20 oral	20 oral	20 oral
B *b*	1 oral	11 oral	13 oral	19 oral 1 both
Check	5 oral			Long 6 oral 1 both Median 5 oral 1 both Short 8 oral 1 both

After 8 hrs.	24	31	34	48
A *a*	20 oral	20 oral	20 oral	15 oral 5 both
B *b*	6 oral 3 aboral	10 oral 3 aboral 6 both	9 oral 4 aboral? 5 both	11 oral 2 aboral 7 both
Check		Long 3 both 7 oral		

In the first of these tables (in which the *b*-end was oblique), there is no influence on the *b*-end observable: in the second table (in which the *b*-end was square), the effect of the second cutting is clearly seen. It is surprising to find that as long an interval as eight hours appears to be no more effective than an interval, between the two operations, of two or three hours.

In the two following experiments a large number of pieces were used, but the interval between the two operations was only one hour. In the first the *b*-end was oblique; in the second it was square.

After 1 hr.	23	$27^1/_2$	$30^1/_2$	48
A *a*	7 oral	11 oral	12 oral	20 oral
B *b*.	3 aboral	4 oral 5 aboral	4 oral 5 aboral	11 oral 3 aboral 2 both

After 1 hr.	25	29	32	48
A *a*	16 oral	16 oral	16 oral	16 oral
B *b*	1 aboral	5 oral 1 aboral	7 oral 1 aboral	9 oral 1 aboral 3 both 1 ?

Despite the obliquity of the *b*-end in the first of these two tables the effect of the second cutting in very obvious. It is less so, perhaps, in the second table, in which the *b*-end was square, but it is still evident.

All of these tables taken together show, I think, that the development of the heteromorphic, or aboral (proximal) polyp can be hastened by cutting a piece in two in the middle; and under these circumstances the aboral polyp may appear almost as soon as does the oral polyp on the other; i. e., the distal piece. The factors in the proximal piece, *B—b*, appear to be so nicely balanced after the second operation, that sometimes the one, sometimes the other predominates; and in doing so may act as a check, if only a partial one, on the factor at work in the opposite direction.

Provisionally, I offer the following analysis of the results. When a piece of the stem of Tubularia is cut off, both ends, after closing, begin to produce new polyps, but on account of the polarity of the piece the anterior end gets a start. During the development going on at this end, the formation of the aboral polyp is held in check; but after the distal (or oral) polyp has once been formed, the influences acting at the aboral cut-end may be strong enough to produce the aboral polyp. Another result that I have often obtained tends to confirm this point of view; viz., that short pieces, 2 to 10 mm in length, less often produce aboral polyps than longer pieces. I interpret this to mean that the nearness of the aboral end to the polyp accounts for the holding in check of the aboral polyp. If, however, the piece is long this influence is insufficient to hold back the development of a polyp on the aboral end.

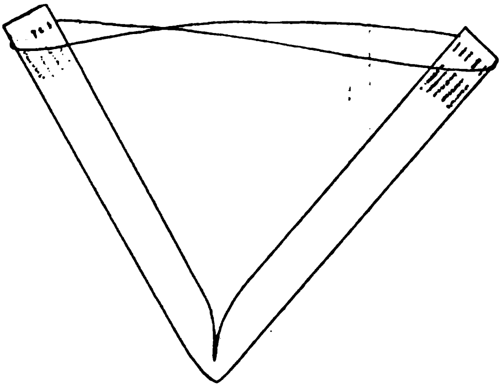
When a piece is cut in two in the middle one, two, three, or more hours after its ends have closed, as in the preceding experiments, the influence of the oral end is temporarily removed, and the aboral end, which now has a start on the new oral end, may gain the ascendency and be the first to produce a polyp. Often, however, the polarity of the piece is sufficiently strong to give the precedence to the influences acting on the oral end. When the two influences are equally balanced, two hydranths may simultaneously develop. Whether under the last conditions the opposing influences may sometimes hold each other in check, so that nothing develops, can not be determined from the evidence at hand.

The results appear also to indicate (but not with sufficient definitenes to make it certain) that when the aboral *b*-end is oblique it less often gives rise to an aboral polyp, after the second cutting, than when the same end is cut off squarely. If this proves to be a fact, it may be due to the greater length of time required for the closure of an oblique surface, or more probably to the greater difficulty in the formation of an oblique anlage.

The next series of experiments have an important bearing on the conclusion reached in this section; and, in fact, without knowledge of the following results the preceding conclusions would scarcely have been warranted.

The Hastening of the Aboral Development by Bending the Pieces.

This experiment was suggested by a result which Vöchting obtained with plants. He found that if a piece of a willow stem is bent in the middle, and suspended in a moist atmosphere, the halves behave in some respects as though they were isolated pieces. I have suggested[1]) that the result might be due to a disturbance of the tensions in the tissue of the meristem, in consequence of which the influences regulating growth (which is also, according to my view, regulated by the conditions of tension in the tissues) are interferred with in the region of the bend.



K

Driesch has shown in Tubularia that by closing the oral end the aboral hydranth develops sooner than when the oral end is open. I wished to see if by bending a piece of the stem the development of the aboral hydranth might also be hastened despite the fact that an oral hydranth is forming at, or about the same time, and also despite the fact that the living tissue is continuous.

It is not difficult to bend long pieces and to hold them in this position, as shown in Fig. *K*, by means of a loop of thread. In nearly all cases the coenosarc remained continuous, although in the region of the bend it was much compressed on one side and extended on the other.

The experiment was repeated a number of times, and always with the same result; viz., the development of the aboral hydranth was hastened. The following four tables give further details. Each of the four vertical series represents consecutive observations on the same pieces.

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[1]) »Regeneration« page 81.

Time	Results
23 hrs.	1 both ends 1 - - 3 one end (check only one end)
25$^3/_4$	3 both ends 2 one end
28	3 both ends 2 one end check 8 oral 1 both
48	4 both 1 one check 6 one end long 4 both short 4 one
72	4 both ends 1 one end check 6 one end long 4 both ends short 4 one end

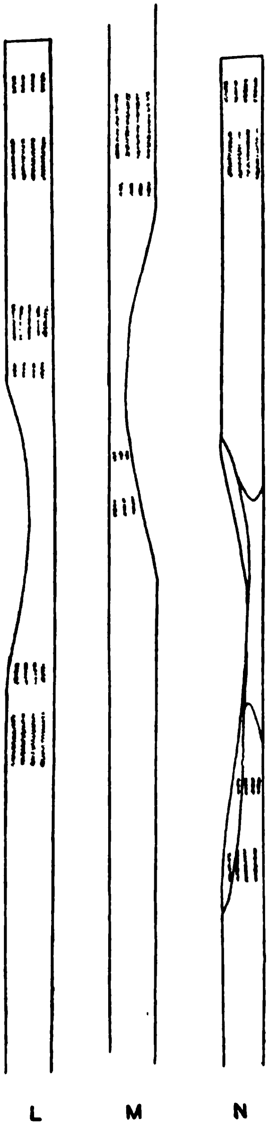
Time	Results
23 hrs.	4 one end check 5 oral
25	3 both ends
42	5 both ends 2 one end check long only one end
48	5 both ends 2 one end
66	6 both ends 1 one end check long 5 one, 3 both

Time	Results
19 hrs.	1 both ends 3 one end 2 none check, 5, all oral
21$^3/_4$	2 both ends 2 one end 2 none
24$^1/_2$	5 both ends 1 one end
27	5 both ends 1 one end
48	6 both ends check long 5 one end. check short 5 one, 1 both.

Time	Results
27 hrs.	3 both ends 3 one 1 none
30$^1/_2$	3 both ends 3 one end 1 none
48	same

These results show that the bending has an unquestionable influence on the development of the aboral hydranth. When compared with the check, unbent pieces the aboral hydranths appear very much sooner. As a rule the oral hydranth begins to appear before the aboral one, but since the original polarity of the piece must be reversed in the latter case this difference is explicable.

The Formation of Two Hydranths after the Removal of a Lateral Piece of the Stem.



By means of a sharp pair of small scissors it is possible to cut out a piece in the middle of the stem as shown in Figs *L*, *M*, *N*. The coenosarc closes over making a smaller tube — hour-glass shaped — in the region of the cut. In a number of cases I have found hydranths developing on each side of the thin part of the tube as shown in Figs. *L* and *M*. They are turned towards each other, and the thinner connecting region is common to the proboscides of the two. Other hydranths may, of course, develop at the squarely cut ends of these long pieces (Fig. *N*).

The main interest attached to this result is that, although the material is continuous between the two parts, nevertheless two hydranths develop. It should be noticed that owing to the closing of the piece the tensions in the different regions may be much altered.

When the two hydranths are nearly formed the material in the thin, intermediate region begins to draw away towards the two hydranths, until finally the connection is broken and the two heads emerge.

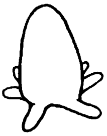
In one case, not figured, the lateral cut lay near the oral end of the piece, and it is interesting to note that a hydranth without a stalk developed, in the oral part its oral end being turned toward the thin region, i. e., in a proximal direction.

The influences bringing about the development under the conditions of this experiment appear to be the same as when the ends themselves are cut off squarely or obliquely. There is a free exposure of coenosarc on the two ends of the hour-glass-shaped cut-region, and this determines the development of new hydranths. The connection of the two ends by a narrower region does not prevent the result. It should be noticed, however, that when only a small part of the side is cut off the development of one, or of two hydranths does not occur.

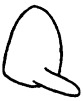
The Ultimate Limit of Reduction of Incomplete Structures.

In several preceding papers I have described some of the different kinds of incomplete structures that are obtained from short pieces. The greatest reduction that I obtained was a proboscis with its circle of distal tentacles. Whether the number of tentacles was less than normal was not determined.

During the course of the present work I have obtained several times very small pieces that showed a reduction in the number of distal tentacles, as shown in Figs. *O* and *P*. In one of the cases, Fig. *P*, there is but a single tentacle.



O



P

It would be difficult to recognize a proboscis without tentacles as a definite structure unless sections were made, and a mouth opening demonstrated, but if the tentacles may be reduced to a single one there can not be much doubt that a proboscis without any tentacles might develop.

This remarkable and almost unique phenomenon in Tubularia of the formation of incomplete, distal structures has been very puzzling, but now, I think, it can be explained. We know that the material of the stem is totipotent. We have evidence showing that it is more difficult for small pieces to produce reduced whole structures than for larger pieces to produce full-length hydranths. At least we may judge so from the longer time that it requires reduced structures to develop. Why a partial structure is sometimes formed in preference to a whole one of reduced size we have not till now been able to explain, but the two points mentioned above taken in connection with the fact, which all observers have recorded; viz., the close union between the coenosarc and perisarc, give us, I believe, the key to the situation.

The diameter of the piece remaining the same as that of the original stem, this factor determines the formation radially of full sized structures, and since the differentiation starts at the free distal end the radial influence that makes for full sized structures predominates over the conflicting influence that tends to reduce the anlage longitudinally to a structure proportionate to the length of the piece. Here then we find in the unique feature of the tubularian stem; viz., the union of perisarc and coenosarc, and the development of the new hydranth within the old tube, the explanation of the frequent formation of incomplete structures instead of smaller ones of proportionate size in all directions.

Summary.

1) The regeneration of short pieces of the same stem of Tubularia indicate that there are individual differences in different stems which may be connected with the relative thickness of the coenosarc. The difference in thickness of coenosarc may also account, in part, for the different behaviour of different regions of the same stem.

2) Alternately long and short pieces of the same stem show that there is no relation in particular between double structures and single ones. It appears, however, that in very short pieces there are two nearly equally balanced and opposing factors; viz., the reducing factor that tends to regulate the size of the oral hydranth-forming-region to that of the piece as a whole, and the influence from the aboral cut-end tending to produce a hydranth there. A third factor may be also recognized; viz., the disproportion of the radial diameter, which, owing to the union of coenosarc and perisarc, leads to the production of full-sized structures. According to which of these factors predominates there results, a) longitudinally reduced, proportionate structures, or b) double structures, or c) incomplete structures with organs that are formed full size.

3) Very short pieces with one end buried in the sand produce whole or incomplete structures at the free end, regardless of whether this is oral or aboral. Double structures rarely develop.

4) The number of tentacles produced by a piece is principally determined by the circumference of the piece. In very short pieces the hydranth anlage may be greatly reduced lengthwise without a corresponding reduction radially in the number of tentacles. This is explained as the result of the close union of the coenosarc and perisarc, which keeps the coenosarc extended to the full-size. The result shows that regulative reduction of the organization may take place in one direction without affecting the other. A simple physical

factor; viz., the union of coenosarc and perisarc, is responsible for the lack of regulation in the radial direction.

5) An oblique proximal end does not cause the formation of an oblique anlage in the distal hydranth, even when the latter extends into the oblique region (Figs. *C*, *E*). The tentacles may be less developed on the oblique surface where the oblique end has closed over.

6) Single structures may be produced by allowing the oral end to close; then after one, two, three, or more hours, cutting off a small piece of this end (Figs. *G*, *a*). The result seems to be due to the start which the cut-end is given over the other end.

7) If a long piece of the stem is cut off and the ends allowed the close (Figs. *H*, *I*, *J*), and if then after one, two, three, or more hours the piece is cut in two in the middle, the development of the proximal hydranth (at *b* in Figs. *H*, *I*, *J*) of the proximal piece (*B*—*b*) is often hastened. This appears to be due to the start in development given to the proximal end as compared with the distal end of the same piece.

8) By bending a long piece (Fig. *K*) the formation of the proximal hydranth is greatly hastened, although the coenosarc of the bent piece is continuous at the bend. It is suggested that the result is due to the removal of the inhibitory effect, which the developing distal hydranth has on the development of the proximal hydranth; perhaps by altering the system of tensions of the piece.

9) If a large piece is cut out of the side of the stem, as shown in Figs. *L*, *M*, *N*, a hydranth may develop on each side of the cut-region, although after the coenosarc heals over the cut-surface the two sides of the cut-region are continuous. The exposure of the oblique ends of the coenosarc accounts for the result.

10) The smallest incomplete structure as yet found is a proboscis with one distal tentacle, Fig. *P*.

11) The most peculiar phenomenon in the regeneration of Tubularia; namely, the formation of distal incomplete structures, is explained as the outcome of the unique condition in the stem; viz., the union of the coenosarc and perisarc. As a result the coenosarc is kept full-sized, and, in consequence, the tendency to form distal full-sized structures is stronger than the tendency to reduce (i. e. to regulate) the new structure radially to one proportionate to the length of the piece.

Zusammenfassung.

1) Auf einander folgende kurze Stücke desselben Tubularienstammes zeigen, dass individuelle Unterschiede bei verschiedenen Stämmen bestehen, die möglicher Weise mit der relativen Dicke des Cönosarks zusammenhängen. Dieselben Dickenunterschiede des Cönosarks mögen theilweise das verschiedene Verhalten der verschiedenen Bezirke desselben Stammes verschulden.

2) Abwechselnd lange und kurze Stücke desselben Stammes zeigen, dass dabei keine einseitig beschränkte Beziehung zwischen doppelten und einfachen Strukturen besteht. Immerhin scheinen in sehr kurzen Stücken zwei nahezu einander die Wage haltende entgegengesetzte Faktoren thätig zu sein: der reducirende Faktor, welcher die Größe des hydrantbildenden oralen Bezirks entsprechend der Größe des Stücks als Ganzen zu reguliren strebt, und der Einfluss des aboralen Schnittendes, der dort einen Hydranten zu bilden strebt. Man kann noch einen dritten Faktor erkennen, nämlich die zu Missverhältnis führende Änderung des radialen Durchmessers, der aus der Vereinigung von Cönosark und Perisark herrührt und der die Entstehung von Bildungen in normaler Größe begünstigt. Entsprechend dem Vorherrschen des einen oder anderen von diesen Faktoren ergiebt sich: a) der Länge nach verkürzte, in sich proportionirte Bildungen, oder b) Doppelbildungen, c) unvollständige Bildungen mit Organen in voller Größe.

3) Sehr kurze Stücke, deren eines Ende im Sande vergraben ist, bringen ganze oder unvollständige Bildungen an dem freien Ende hervor, ohne Rücksicht, ob dieses das orale oder aborale ist. Doppelbildungen gelangen selten zur Entwickelung.

4) Die von einem Stück hervorgebrachte Tentakelzahl hängt hauptsächlich mit dem Querumfange des Stücks zusammen. In sehr kurzen Stücken kann die Hydrantanlage der Länge nach eine erhebliche Reduktion erfahren, ohne dass in radialer Richtung eine entsprechende Reduktion der Tentakelzahl stattfände. Es ergiebt sich dies als Folge der engen Vereinigung von Cönosark und Perisark, welche das Cönosark zu voller Größe ausgedehnt erhält. Es zeigt sich somit, dass eine regulatorische Reduktion der Organisation nach einer Dimension stattfinden kann, ohne dass dies nach den anderen der Fall zu sein braucht, Ein einfacher physikalischer Faktor, nämlich die Vereinigung von Cönosark und Perisark, ist für den Regulationsmangel in radialer Richtung verantwortlich zu machen.

5) Ein schräges proximales Ende wird nicht Veranlassung zur Entstehung einer schrägen Anlage des distalen Hydranten, selbst wenn sich der letztere bis in den schrägen Bezirk erstreckt (Fig. *C*, *E*). Die Tentakeln können an der schrägen Oberfläche dort in der Entwickelung zurückbleiben, wo das schräge Ende zum Verschluss sich herüberlegte.

6) Einfachbildungen kann man dadurch hervorbringen, dass man dem oralen Ende gestattet, sich für ein, zwei, drei und mehr Stunden zu schließen, und dann ein kleines Stück von diesem Ende abschneidet (Fig. *Ga*). Die Ursache des Ergebnisses scheint der Vorsprung zu sein, welchen das Schnittende vor dem anderen hat.

7) Wenn ein langes Stammstück abgeschnitten und dem Ende erlaubt wird, sich zu schließen (Fig. *H*, *I*, *J*), und wenn dann nach einer, zwei, drei und mehr Stunden das Stück in der Mitte entzweigeschnitten wird, so wird die

Entwickelung der proximalen Hydranten (bei *b* in Fig. *H*, *I*, *J*) des proximalen Stücks (*B*—*b*) oft beschleunigt. Dies scheint auf der dem proximalen gegenüber dem distalen Stück desselben Stücks gegebenen Entwickelungsbeschleunigung zu beruhen.

8) Durch die Biegung eines langen Stücks (Fig. *K*) wird die Bildung des proximalen Hydrants erheblich beschleunigt, obgleich das Cönosark des gebogenen Stücks an der Biegung nicht zertrennt ist. Es ist anzunehmen, dass dieses Ergebnis dem Fortfall des behindernden Einflusses zuzuschreiben ist, welchen der sich entwickelnde distale Hydrant auf die Entwickelung des proximalen Hydranten hat; vielleicht beruht dieser Fortfall auf Störung der Spannungsverhältnisse des Stücks.

9) Wird ein großes Stück aus der Seite des Stammes geschnitten, wie Fig. *L*. *M*, *N* zeigen, so kann sich an jeder Seite des Schnittbezirks ein Hydrant entwickeln, wenn auch die zwei Seiten der Schnittregion zusammenhängen, indem sich das Cönosark über die Schnittfläche zusammenbiegt. Das Freistehen der schrägen Enden des Cönosarks ist für dieses Ergebnis verantwortlich.

10) Die kleinste bis jetzt gefundene unvollständige Bildung ist ein Rüssel mit einem distalen Tentakel (Fig. *P*).

11) Die eigenthümliche Erscheinung bei der Regeneration der Tubularia, nämlich die Entstehung distaler, unvollständiger Bildungen, erklärt sich als die Folge der eigenartigen Vorbedingung, welche der Stamm dafür liefert, nämlich der Verschmelzung von Cönosark und Perisark. In Folge davon behält das Cönosark seine volle Ausdehnung und in Folge dessen ist die Tendenz zur Bildung distaler Strukturen voller Größe stärker als die Tendenz zur radialen Reduktion, d. h. Regulation, der Neubildung auf eine der Länge des Stücks entsprechende Größe.

Further Studies on Regeneration in Hydra viridis.

By

Helen Dean King.

With Plates ✓IV—✓VI.

Eingegangen am 22. November 1902.

The following experiments on regeneration in Hydra viridis are a continuation of those recorded in a previous paper (King, 2). They were carried on during the winter of 1901—1902, under the direction of Professor T. H. Morgan to whom I am indebted for many helpful suggestions.

Methods.

The operations were made in a shallow dish containing a thin layer of paraffine which forms an excellent surface for the attachment of the polyps, and also for cutting. The dish was kept nearly full of fresh water, and the polyps were not removed from it until the immediate effects of the operation had passed, when they could be transferred into other dishes without fear of injuring the tissues or of separating the components of a graft.

In making lateral grafts, the paraffine method invented by Rand (8) was used at first; but so much time was required to make the proper grooves in the paraffine and to keep the components of the graft from pulling apart, that this method was soon discarded. Lateral grafts were then made by uniting pieces of hydra on fine glass thread, a method that I had used successfully in my former experiments with end-to-end grafts. In making lateral grafts by this means, however, the glass thread had to be pushed through the walls of the stock hydra. In many cases this caused a considerable laceration of the tissues, and, therefore, although much less time was required to make the grafts and a larger per cent. of them were successful than with the paraffine method, this method was also given up. It was discovered, by chance, that if the freshly cut surfaces of two pieces of hydra are brought into good contact, they will readily unite and form a perfect graft without any attempt being made to hold the parts together for a considerable period of time. Acting on this suggestion, the greater number of experiments in lateral grafting were made in the following manner: After a polyp had become firmly attached to the paraffine, a break was made in the body wall either with a sharp scalpel or with the point of a needle. Before the edges of the break had time to come together, the freshly cut surface of another hydra was brought into contact with the injured surface of the first polyp, and the pieces were held in place for a moment only. The graft was left undisturbed for two or three hours, and it was then transferred into a small glass dish filled with fresh spring water. If the pieces of hydra did not adhere when they were first brought into contact, they were again put in place and held for a short time. If this second attempt at grafting proved unsuccessful, the pieces were either discarded or fresh surfaces cut, as, except in rare instances, union between parts of different individuals is only possible when freshly cut surfaces are brought together.

Occasionally, instead of making a wound in the body wall of the stock hydra, the point of a small, sharp scalpel was inserted in the mouth of a contracted polyp, and a slit made down one side of the body nearly to the foot. The edges of the slit separated at once so that the polyp appeared, for a short time, as a flat sheet of tissue with the endoderm uppermost. The freshly cut surface of the graft hydra was then quickly brought into contact with the endoderm of the stock, and held in place until the cut edges of the stock rolled up and surrounded it. Most of the experiments in which a piece of a polyp was inserted high up in the stock were made in this manner.

By the above methods, which in a slightly modified form have recently been successfully employed by Hefferan (1), 20—25 lateral grafts can easily be made in the course of an hour, and considerably over 50% of them are usually successful.

In order to maintain a proper oxygen equilibrium, green water plants, Anacharis, Nitella, etc., were always kept in the dishes containing the polyps, and every few days the water was changed and the dishes thoroughly cleaned to prevent contamination by bacterial growths. Small Crustacea and Protozoa were frequently supplied for food, as hydras, when deprived of food for any length of time, decrease greatly in size, lose a number of their tentacles, and become totally unfit for experimental purposes.

I. The Effect of Light on the Regeneration of Hydra viridis.

While studying the effects of temperature on the regeneration of Hydra viridis and of Hydra grisea, PEEBLES (6) also made some experiments in which she tried the effects of monochromatic lights (red, blue, green and yellow), and also of darkness and of diffuse daylight on the process of regeneration. As a result of her experiments she states: »the process of regeneration was in no way influenced by any of the colors«. PEEBLES seems to have been interested only in the effects of temperature and of color on the rate of regeneration of the polyps. As it seemed not impossible that the presence or absence of light might affect the number of tentacles that would regenerate after the removal of the head of a polyp although the rate of the regeneration remained the same in both cases, the following experiments were made to determine this point.

On Jan. 13, 1902, twenty-four hydras were taken from an aquarium and separated into two lots, A and B, each containing 6 seven-tentacled hydras and 6 eight-tentacled hydras. The head of each polyp was then removed by a transverse cut just back of the circle of tentacles. The dish containing lot A was kept on a table in the laboratory so that the polyps were exposed to light, but not to the direct rays of the sun. Lot B was put into a dish covered on the outside with black cloth, and the dish was then shut up in a drawer in the laboratory table as an additional precaution against the penetration of light rays to the polyps. Both lots of hydras were kept in the same room and, consequently, they were under similiar conditions of temperature.

The polyps were examined for the first time on Jan. 16, and in both lots tentacles had appeared. The tentacles on the polyps in lot B seemed fully as well developed as those on the polyps in lot A, thus confirming PEEBLES' result that the rate of regeneration is not affected by keeping the polyps in continued darkness. The hydras were then left undisturbed until Jan. 21, when the number of tentacles that had regenerated on each lot of polyps was counted carefully. The result is shown in the following table.

Table I.

	No. tentacles regenerated					Average
	5	6	7	8	9	
Lot A (exposed to light)		2	5	4	1	7.33
Lot B (kept in darkness)	1	4	6	1		6.58

Before the operation, the average number of tentacles possessed by the polyps was 7.5. In the above table it is seen that the average number of tentacles that has regenerated on each lot of hydras is less than the number originally possessed by the polyps, agreeing, in this respect, with the results obtained by Rand (8) in his study of the relation existing between the original number of tentacles on an individual and the number regenerating after the head of the polyp has been removed. Table I also shows that the individuals kept in darkness have regenerated an average of 0.75 tentacles per hydra less than have those polyps that were exposed to the light. It would seem, therefore, that the presence or absence of light influences the number of tentacles that will regenerate after the removal of the head of a polyp. It might, however, be possible to explain the result as due to individual differences in the size of the polyps in the two lots, as Rand has shown that large individuals tend to regenerate a greater number of tentacles than do small individuals. To determine this point, the new heads that had regenerated on the polyps in both lots were cut off on Jan. 21, close behind the circle of tentacles as in the first operation. The hydras of lot A were then put in the dark and those of lot B were allowed to regenerate in the light, thus reversing the conditions under which they had regenerated in the first instance. If the difference in the number of tentacles that regenerated on the two lots of polyps as shown in Table I was due solely to the larger size of the individuals in lot A, it ought to follow, as a result of the second removal of the heads, that the individuals of lot A, although kept in darkness, will regenerate more tentacles than will the presumably smaller individuals of lot B which are exposed to the influence of the light. On Jan. 28, the tentacles on all of the polyps were counted with the result shown in Table II.

Table II.

	No. tentacles regenerated				Average
	5	6	7	8	
Lot A (kept in darkness)	3	4	4	1	6.25
Lot B (exposed to light)		4	5	3	6.91

In the above table it is seen that the polyps kept in the light (lot B) have regenerated an average of 0.66 tentacles per hydra more than have the polyps of lot A which were kept in darkness. This result would certainly not have occurred if lot A, by chance, had contained larger individuals than lot B and if the size of the individual alone determines the number of tentacles that will regenerate in a given case. It appears, therefore, from the above experiments, that the presence or absence of light determines, to a certain extent at least, the number of tentacles that will regenerate on a polyp after the removal of the head.

On March 21, the above experiment was repeated on twenty-four individuals taken from a different aquarium than were those used in the first case. Again, on April 30, the same operation was made on a third set of twenty-four individuals which had just been brought in from a pond near the laboratory. The results obtained in these last two sets of experiments, since they confirm those obtained in the first set, are summarized together in the following tables. The number of tentacles originally possessed by the polyps is shown in Table III.

Table III.

	Original No. of Tentacles			
	6	7	8	9
No. Individuals	12	16	18	2

The average number of tentacles possessed by the polyps in the beginning of the experiment was 7.20. This is somewhat less than the average for the hydras of the first set where individuals with seven or eight tentacles were especially selected. In the present experiments great care was taken to choose polyps of as nearly the same size as possible in order that the results could in no possible way be influenced by individual differences in the size of the hydras.

The second set of polyps was examined three days after the removal of the heads in order to see if the rate of regeneration in the two lots of the set varied at all. It was again found that the individuals kept in darkness regenerated just as quickly as those exposed to the influence of the light. In the third set, no observations were made on the polyps kept in darkness until the end of a week when the tentacles in both lots were counted. The number of tentacles that had regenerated on all of the 48 polyps one week after the removal of the heads is shown in the following table.

Table IV.

	No. tentacles regenerated					Average
	4	5	6	7	8	
Lot C (exposed to light)	1	2	7	12	2	6.50
Lot D (kept in darkness)	3	11	7	2	1	5.41

In the above table it is seen that the polyps kept in the light have regenerated an average of 1.09 tentacles per hydra more than have those deprived of light. This difference is 0.34 greater than was obtained in the first set of experiments recorded in Table I.

After the removal of the new heads of the polyps by a transverse cut just behind the circle of tentacles, the conditions of the lots were reversed, i. e., lot C was put in darkness and lot D was exposed to the light. The number of tentacles that had again regenerated on the polyps at the end of another week is shown in Table V.

Table V.

	No. tentacles regenerated					Average
	3	4	5	6	7	
Lot C (kept in darkness)	3	10	8	3		4.45
Lot D (exposed to light)		6	5	10	3	5.41

Summarizing the results of the three sets of experiments given in the preceding tables, it is found that a total of 36 hydras with an average of 7.35 tentacles per hydra at the beginning of the experiment, will, if exposed to the influence of light after the removal of the head of each polyp, regenerate an average of 6.91 tentacles per hydra. The same number of polyps with the same average original number of tentacles will regenerate an average of only 5.99 tentacles per hydra when deprived of light during the process of regeneration. The difference of 0.92 tentacles per hydra in the two cases is much too large to be ascribed to mere individual variation in capacity for regeneration, especially as, in two of the three sets of experiments, great care was taken to use individuals of as nearly the same size as possible. After removing the head of each polyp a second time and reversing the conditions under which they were regenerating, so that the polyps previously exposed to the light were now the ones kept in darkness and vice versa, it was found, one week after the operation, that the polyps which had been regenerating in the dark had produced an average of only 5.35 tentacles per hydra, while those which had received light had regenerated an average of 6.16 tentacles per hydra. The difference in the two cases being 0.81.

As the question of variation in the size of the polyps in the different lots can, perhaps, be excluded in considering the results, the obvious conclusion from the experiments would seem to be that continued darkness affects the regeneration of Hydra viridis in that it prevents the formation of as many tentacles as will be produced if the hydra is exposed to the light during its regeneration. Hydra viridis, as is well known, is positively heliotropic. It is possible that the presence of light is necessary to enable the cells of the polyp to properly perform their nutritive functions, and that darkness may so interfere with the nutrition of the polyp that the regenerative processes, although they are not delayed, are less vigorous than they are under normal conditions.

While the above investigations were in progress, a number of normal polyps were kept in continued darkness for some two months. They were supplied with food and only examined every two weeks when it was necessary to give them fresh water. No marked changes appeared in the polyps during the time they were kept under observation. They maintained their normal size and color; the number of tentacles remained the same, or in some few cases increased; and in several instances buds were produced. It would seem, therefore, as if continued darkness has no effect on normal individuals, although depriving the polyps of light for a much longer period, an experiment which I hope to try at some future time, may show changes in the structure of the cells, if not in the external characters of the polyps.

II. Experiments in Grafting.

A. Lateral Grafts.

Rand (9) made a number of interesting experiments in which he inserted a piece of one polyp, the »graft«, into the side of another polyp, the »stock«, producing compounds which he called »lateral grafts«. Rand used Hydra viridis for most of these experiments, not only because of its abundance, but also because of the quickness with which it regenerates. From his experiments, Rand concluded that, with few exceptions, lateral grafts do not persist as permanent abnormalities, but that regulation to a normal form is produced in most cases by »a slow migration of the graft down the trunk of the stock until graft and stock arise directly from a common foot. A constriction then slowly forms between graft and stock, which finally separate. Sometimes the graft constricts and separates from the stock before the migration to the foot is completed«. In case the graft was very small and without tentacles, Rand found that it failed to maintain its individuality and was absorbed by the stock without regenerating.

Lateral grafts similar to those of Rand have recently been made by Hefferan (1) who used Hydra fusca, Hydra grisea, and Hydra monoecia as well as Hydra viridis in her experiments. She finds that the process of regulation to a normal form differs in different species of Hydra. Hefferan's summary of a part of her work is as follows:

»Regulation of lateral grafts in Hydra fusca is usually a double process of migration of the graft, and of fusion as the result of tension, i. e. the graft tends to migrate towards the head end of the stock until the head ends of graft and stock are equal in length, when fusion gradually brings them together. There is, however, a level of insertion somewhere in the aboral $^1/_5$ of the stock, below which a graft will move downward and constrict off.

Regulation of lateral grafts in Hydra viridis is usually a process of downward migration, constriction and separation at the foot of the stock. Occasionally a graft inserted very near the oral end of the stock will persist for sometime and finally fuse as in Hydra fusca.

The difference in the behavior of lateral grafts in Hydra fusca and Hydra viridis is probably due to a difference in the diameters of the cylinders which form them, and to the action of capillarity.«

Many of the following experiments in lateral grafting were suggested by the work of Rand. They were undertaken, primarily, for the purpose of obtaining additional cases of heteromorphosis, and to ascertain, if possible, why in some cases a graft moves to the foot of the stock before constricting off, while in other cases it develops a foot on its aboral end in the course of a few days after the operation and then soon becomes a separate individual, having undergone little, if any, downward migration towards the aboral end of the stock.

I have shown, in my previous paper, that when individuals of decidedly different shades of green are united together, the tissues of each component can be readily distinguished for a number of days after the graft is made; in some cases two or three weeks elapse before the compound becomes the same shade of green throughout its entire extent. In many of the following experiments, therefore, very light and very dark green individuals were united together in order to ascertain the part played by each component in the subsequent changes that took place.

Only full grown polyps were used in making these experiments, and, with but the very few exceptions which are noted, individuals of average size and with from 6—8 tentacles were selected in order that the results could not be influenced by the use of individuals that might be considered abnormal in the slightest degree. The sign + is used in the figures to designate the oral end of a polyp, and the sign — to indicate the aboral end; if arrows are used they point in every case towards the oral end of the polyp. Where individuals of different shades of green were grafted together, the darker colored tissue is dotted in the figures to distinguish it from the lighter colored tissue.

1. Experiments to determine the manner of separation of Graft and Stock.

Rand states that in nearly all lateral grafts separation of the graft from the stock takes place through »a downward migration of graft upon stock until both arise immediately from the original foot of the stock hydra. A constriction then appears between graft and stock and a foot is formed at the base of the graft. As the constriction deepens the connection between the two polyps becomes drawn out to a slender thread, in which both body layers are present for a time. The thread finally parts leaving two normal hydras«.

In three of the twenty-three experiments made by RAND, a foot developed on the aboral end of the graft in the course of a few days after the operation, and the migration of the graft down the trunk of the stock was either prevented entirely, or else only a very slight downward movement took place. Separation of the two components of the graft occurred through a gradual constriction of the graft from stock and was completed in a much shorter time than in the other cases.

HEFFERAN's experiments on lateral grafting in Hydra viridis show a process of regulation very similar to that taking place in the lateral grafts made by RAND as she found that »in every case migration towards the foot and final constriction and separation occurred either before or after reaching the foot«.

There is, apparently, no reason why in some of these experiments a foot should develop on the aboral end of the graft and the graft constrict off from the stock a few days after the operation, while in similar experiments a downward movement of the graft to the foot of the stock takes place before the separation of the polyps is effected. Can the difference be due, perhaps, to an imperfect union of the graft and stock in some cases, or is the size of the graft and its location in the stock of importance in determining which of these modes of separation shall take place?

Series I. In this set of experiments, the graft consisting of two-thirds or less of the anterior end of a polyp, was inserted into the side of another individual either just below the circle of tentacles or directly on the hypostome. In the latter case, the compound was made by splitting the oral end of the stock and bringing the graft directly in contact with the endoderm.

Experiment I. On Jan. 10, the foot was removed from a dark green polyp bearing seven tentacles and the anterior two-thirds of the body was grafted into the side of a large, light green, eight-tentacled hydra just below the circle of tentacles (Fig. 1). During the two following days the compound was examined frequently and the stock was usually found to be bent over considerably, although the axis of the graft remained nearly at right angles to that of the stock. On Jan. 15, the graft was still attached to the stock just below the circle of tentacles and showed no signs of a downward movement towards the foot of the stock. Up to this time the line of demarcation between the two components was sharp and distinct, and there had been no intrusion of the light green tissue of the stock into the dark green tissue of the graft or vice versa. On Jan. 19, a slight projection was noticed at the aboral end of the graft and the point of this projection adhered to a needle when touched. Two days later a constriction between the graft and the stock had appeared and the graft was attached by the foot which had developed at its aboral end. On Jan. 28, the compound appeared as in Fig. 2. The axis of the graft was no longer at right angles to the axis of the stock, and the two polyps were connected only by a narrow thread of substance. Final separation occurred on Jan. 31, when a dark and a light green hydra, each apparently normal in every respect, were found attached side by side.

Five other experiments, similar to the preceding, gave a like result. The time required for a complete separation of the components of the graft varied from 14—22 days, and there was no apparent movement of the graft towards the aboral end of the stock in any case.

Experiment 2. A little less than one-half of the anterior end of a dark green polyp was grafted into the side of a light green polyp just below the circle of tentacles. The operation was made on Jan. 9. The following day the compound was found to maintain an upright position, and as the stock had become bent where the graft was attached, the axis of the graft and that of the upper part of the stock formed nearly equal angles with the axis of the common trunk thus producing a Y shaped structure (Fig. 3). The arms of the Y were of equal length on Jan. 16, showing that the graft had migrated a short distance down the trunk of the stock. This downward movement of the graft could not be attributed to a splitting of the stock at the angle of the Y, because, owing to the difference in the color of the two components, it was seen that the light colored tissue of the stock ended abruptly where the graft joined it, and that it did not extend up into the trunk of the graft as it ought to do if a splitting of the stock instead of a downward migration of the graft had taken place. Fig. 4 shows the condition of the graft on Jan. 30. The axial relations of the part are the same as in the previous figure, but the arms of the Y are decidedly longer. There is still sufficient distinction between the colors of the components to show conclusively that the increase in the length of the graft has not been caused by an absorption of a part of the stock; but it is either the result of growth or, possibly, it is due to a rearrangement of the tissues of the graft itself, as Morgan (4) has shown that even a small ring cut from the body of a hydra is capable of elongating until it obtains the typical proportions of a normal polyp, without the apparent formation of any new tissue at the cut ends. On Feb. 10, the compound appeared as in Fig. 5, the common trunk being but slightly thicker than the diameter of either polyp. As the graft head had six tentacles and the stock head had seven tentacles, the two components were readily distinguished, although by this time all distinction in color between the graft and stock had disappeared. A greater part of the time the two polyps were extended out in opposite directions and each seemed striving to pull away from the other. By Feb. 18, the constriction between the two polyps had progressed so far that only a slender thread of ectoderm held the components together (Fig. 6). Final separation of the polyps took place on Feb. 21.

The eight other grafts that were made in the same manner gave practically the same results. Separation of the graft from the stock occurred in every case at the foot of the stock in from five to seven weeks after the operation was made.

In comparing the different modes of separation of the graft from the stock in experiments 1 and 2, it will be noted that, when more than one-half of the anterior end of a polyp is grafted into the side of another polyp near the oral end, the axial relations assumed by the parts of the compound very soon after the operation are very different from those that are found when the graft consists of less than one-half of a polyp. In the first case, the axis of the stock does not become bent at the place of union of the graft and stock, but remains nearly straight during the entire time of the experiment. The graft develops a foot on its aboral end and separates from the stock in a comparatively short space of time, having undergone little, if any, downward movement towards the posterior end of the stock. In the second case, where the axis of the stock above the place of attachment of the graft becomes bent so that the compound appears as a Y shaped structure, there is a gradual downward migration of the graft to the aboral end of the stock where the final separation of the two polyps occurs.

As less than one-half of the anterior end of a polyp, when used as a graft, is capable of maintaining its individuality and finally separating from the stock, it seems strange that the quicker method is not always employed to separate the components. Possibly the stock, on account of its larger size, has sufficient influence over the fate of the graft to delay the final separation; yet as both components of the graft seem constantly striving to pull away from each other. it would seem that the larger piece ought to aid instead of hinder this separation.

The downward movement of the graft to the foot of stock in the above experiments cannot be due to a splitting of the stock, as HEFFERAN considered to be the case in a somewhat similar experiment that she made. For, as shown in experiment 2, as long as the distinction in color between the two parts of the graft remained, the line of union between the graft and the stock was well marked and there was no intrusion of the tissues of the stock into the graft. The downward migration is a movement of only the graft down the trunk of the stock, and it does not include a movement of some of the tissues of the stock also.

RAND has suggested that the migration of the graft down the trunk of the stock might be due to the purely mechanical effect of gravity. Experiments which I made to determine this point (KING, 2), showed that the separation of the parts of a double-headed hydra takes place even when the polyp is kept in an inverted position and, therefore, that the process is independent of the action of gravity. I have suggested that the cause of the separation of the parts of a double-headed polyp may be found »in the constant struggle of each part to free itself from the attachment and maintain its own individuality«. I see no reason why the separation of the graft from the stock in the above experiments cannot also be explained in the same way. Tension exerted on the place of union of the two components by the constant straining of the two heads in opposite directions would tend to cause a weakening of the cohesion of the cells in this region. When the two arms of a Y shaped compound are of nearly equal length and have the same angle with the axis of the common trunk, the pull would be nearly equal from both sides. The result would be a gradual lengthening of the arms and a corresponding decrease in the length of the common trunk until, finally, a separation of the components at the foot of the stock would take place whether the compound was inverted or kept in an upright position. When, on the other hand, the axial relations of the part of the compound are such that the axis of the stock remains nearly straight while the axis of the graft hydra forms an angle with it, the tension at the place of union of the graft and stock is necessarily different from what it is in the first case and the effect on the tissues seems to be such that the graft is able to exert its individuality in a much shorter time, and to constrict off before it has undergone any perceptible downward migration towards the aboral end of the stock.

In a number of grafts made as were those in the previous experiments, the components were only partially united at first. Invariably, in such cases, a constriction appeared between the graft and the stock and a foot developed on the aboral end of the graft in from two to four days after the operation. Separation of the parts of the compound occurred in the course of a week in every case and there was no apparent movement of the graft towards the aboral end of the stock. Owing, doubtless, to the imperfect union, the graft in these experiments was very soon able to exert its individuality and separate from the stock. As no definite conclusions could be drawn from these grafts, no attention was paid to the axial relations of the component parts.

Experiment 3. The head of a large, dark green, eight-tentacled hydra was cut off close behind the circle of tentacles on April 8, and grafted on a light green, eight-tentacled polyp just below the hypostome (Fig. 7). The experiment was made to see whether the two heads would fuse or whether the graft would grow at the expense of the stock and finally separate from it. Grafts of this character are very difficult to make as, owing to the small size of the graft and the tendency of the tentacles to contract, it is almost impossible to keep the parts in good contact until they have completely united.

By April 12, it was evident that the two heads were in the process of fusion as the hypostomes were much closer together than they were when the operation was first made. Fusion was completed by April 16, and the compound had but a single mouth opening surrounded by 16 tentacles. Eight of the tentacles, evidently the ones originally belonging to the graft head, were considerably darker in color than the rest. This abnormal polyp was kept for several weeks in order to find out how the regulation to a normal number of tentacles would be brought about. All distinction in the color of the tentacles disappeared in the course of a few days after fusion was completed, so that it was not possible to follow the fate of either set of tentacles. On April 21, six of the tentacles were found to be fusing in pairs from their bases up. They thus presented the branched appearance described by Parker (5) and Rand. At this time, four other tentacles were observed to be somewhat smaller than the rest, and it was evident that these tentacles were undergoing a gradual process of absorption. On April 26, the polyp had but nine tentacles and this number remained constant during the rest of the time the hydra was kept under observation, a period of about three weeks.

Four other grafts of this nature were successful. In each case the two heads fused completely in the course of a week forming a polyp with a single hypostome and with 13, 14, 16, 17 tentacles respectively. Regulation to the normal number of tentacles was brought about, as in the preceding case, by a fusion of some of the tentacles and an absorption of others until the number was brought within the limits of normal variation.

Attempts to graft one of these multi-tentacular heads on to another polyp were not successful as the component always separated in the course of twenty-four hours, although at first they appeared to be firmly united.

The above experiments seem to show that when the head only of a polyp is grafted very close to the oral end of another polyp, the graft, although it bears tentacles, is not destined to separate from the stock, but to become incorporated into it so that eventually a normal polyp is formed from the compound. In lateral grafts of Hydra fusca, HEFFERAN has noted a tendency of the graft to migrate towards the oral end of the stock, and there to fuse completely with it, the abnormal number of tentacles being subsequently reduced by fusion or absorption as is the case in Hydra viridis. This method of regulation in lateral grafts of Hydra fusca always takes place when the graft is inserted above the aboral one-fifth of the stock. Below this point the graft moves downward and constricts off from the aboral end of the stock. HEFFERAN makes the statement for Hydra viridis that »occasionally a graft inserted very near the oral end of the stock will persist for some time and finally fuse as in Hydra fusca«, although the details of the experiments on which this statement is based are not given in the text of her paper.

The above experiments seem to contradict the statement made by RAND that »if the graft bears tentacles when grafted, or if it regenerates tentacles after the operation, it is destined to separate from the stock . . . the fate of the graft depends upon its degree of specialization«. As a result of my experiments, I have found that the fate of a graft depends, not upon its degree of specialization, but primarily on its size and also, to some extent, on its position in the stock. If more than the head of a polyp is grafted near the oral end of the stock, the component parts invariably separate sooner or later. Experiments to be described later will show that the same rule holds good for large grafts inserted anywhere along the trunk of the stock. A small piece of any part of the body wall of a polyp will be absorbed by any part of the stock, but the head of a polyp can be incorporated only into the oral end of the stock.

Series II. In this series of experiments the anterior part of a polyp was grafted at or very near the middle of another polyp. The details of one of these experiments is as follows:

Experiment 1. On March 31, about two-thirds of the anterior end of a six-tentacled polyp was inserted into the side of a seven-tentacled hydra at the middle region as in Fig. 8. A short time after the operation, the axis of the graft was found to be almost at right angles to the axis of the stock. On April 3, the axial relations of the components were the same, but the graft hydra had lengthened somewhat. Whether this increase in length was due to a rearrangement of the tissues of the graft, or to a growth of new tissue, I was not able to determine. At this time, also, a foot had developed at the aboral end of the graft near its line of union with the stock, and a bud had appeared on the side of the graft opposite to the newly formed foot (Fig. 9). The graft had begun to constrict from the stock by April 5, and the constriction deepened so rapidly that on April 10, the two polyps were connected only by a narrow strip of substance in which both body layers were present for some time Fig. 10). No downward movement of the graft towards the aboral end of the stock occurred and the connection between the polyps broke on April 14, so that two normal individuals were produced.

In the four other grafts which were made in this set, the result was the same as in the previous experiment. A foot formed on the aboral end of the graft, and graft and stock separated in about ten days after the operation. No migration of the graft to the posterior end of the stock was found in any case.

Experiment 2. A graft was made on April 7, in which one-halt of the anterior end of a polyp was inserted in the middle of another polyp. This experiment was similar to the preceding except that the graft was somewhat shorter. Soon after the operation it was noticed that the stock had become bent where the graft joined it. This resulted in the formation of a Y shaped compound in which the axes of the arms formed equal angles with the axis of the common trunk. The arms of the Y were about the same length, but the graft could readily be distinguished from the stock on account of its darker color and because it had but six tentacles while the stock head had eight. During the two weeks following the operation there was a gradual migration of the graft towards the foot of the stock; the arms of the Y, however, kept their same axial relations with the axis of the common trunk. On April 30, the two polyps were attached at the foot of the stock by a band of substance about the width of one of the hydra bodies above the place of union. The compound then presented an appearance much like that of Fig. 5. The connection between the polyps gradually grew thinner and separation was finally effected on May 16. The manner of separation of the graft from the stock in this experiment was exactly similar to that taking place in the majority of the lateral graft of Hydra viridis made by RAND and HEFFERAN.

The previous experiment was one of fourteen in which from one-fourth to one-half of the anterior end of a polyp was grafted on another polyp near the middle region. In every instance the axis of the stock became bent at the line of union of the two components so that a Y shaped structure resulted in which the axis of the arms formed equal angles with the axis of the common trunk. Subsequently a downward migration of the graft to the foot of the stock took place in each case, and constriction and final separation of the two polyps occurred in this region in from four to six weeks after the operation.

Experiment 3. On the morning of June 4, the head only of a dark green, six-tentacled hydra was grafted into the middle of a light green, seven-tentacled hydra as in Fig. 11. During the day of the operation the compound was examined at frequent intervals and the axis of the graft was always found to be at right angles to the axis of the stock. Two days later, however, the axial relations of the parts of the graft were found to be greatly changed from what they were at the beginning of the experiment. The graft had swung around in line with the axis of the common trunk, while the oral end of the stock hydra was bent sharply where the graft joined the stock and its axis formed an angle of about 45° with the axis of the rest of its trunk (Fig. 12). One June 10, the portion of the trunk of the stock above the point of bending of the stock head was found to have increased somewhat in length (Fig. 13). This increase could not be due to a growth of the graft as, owing to the difference in color between the two components, it was seen that the dark green tissue of the graft head extended only just below the tentacles where it was sharply marked off from the light colored tissue of the stock. The apparent growth of the trunk, therefore, must be attributed to a downward movement of the anterior portion of the stock on its own trunk. On June 12, the head of the stock was slightly constricted where it was bent away from the rest of its trunk and a slight projection was noticed at its aboral end (Fig. 13, *P*). The migration of the anterior part of the stock towards the aboral end of the common trunk had progressed considerably by June 16, and the compound was found to be attached not only by the aboral end of the stock, but also by a new foot that had developed from the projection on the anterior part of the stock (Fig. 14). All distinction in color between the graft and the stock had disappeared by this time, although the graft head could be distinguished on account of its having but six tentacles. On June 28, the two parts of the compound were connected a little above the foot region only by a thin thread of ectoderm which broke at once when touched with a needle. Of the two apparently normal hydras that were produced from the graft, one was formed entirely from the anterior part of the stock, the other from a permanent union of the graft head with the aboral end of the stock hydra.

Only one other experiment like the preceding was successful, and its subsequent history was practically the same as that described above. A similar result was obtained by Rand in one of his lateral graft experiments, where about one-fourth of the oral end of a polyp was inserted below the middle of another polyp. The axis of the graft was found to coincide with that of the common trunk a few days after the operation, and later the upper end of the stock moved downward and constricted off from its own foot end.

Series III. In the experiments made in this group, anterior pieces of hydra of various lengths were inserted below the middle of another polyp and the fate of the graft determined.

Experiment I. On May 23, the upper half of a six-tentacled hydra was grafted into an eight-tentacled polyp near the aboral end (Fig. 15). In the course of a few hours after the operation, the stock became bent where the graft was united with it and thus a Y shaped structure was formed as in previous experiments. After a gradual downward movement of the graft to the foot of the stock, constriction and final separation took place in this region.

In five similar experiments the history of the component parts was the same as in the preceding case.

Experiment 2. About one fourth of a dark green, six-tentacled hydra was grafted near the foot of a light green polyp bearing seven tentacles (Fig. 16). The operation was made on May 5. During the following week the graft grew somewhat longer, the increase in length being due to a growth or to a rearrangement of the tissues of the graft alone as none of the light green tissue of the stock extended into it. The axis of the stock soon became bent so that the compound appeared Y shaped and the graft moved to the foot of the stock. On May 13, when all distinction in color between the parts of the graft had disappeared, the two components were found to be united in the aboral region by a band of substance slightly narrower than the diameter of either hydra trunk, and they were attached to the bottom of the dish by a single, broad, flat foot (Fig. 17). Gradual constriction between the polyps took place through the middle line, so that, when final separation occurred on May 20, each polyp received a part of the original foot of the stock.

Six other similar experiments gave a like result as constriction and separation of the polyps occurred at the foot of the stock in about two weeks after the operation. In only two cases, however, did each component of the graft receive a part of the foot of the stock when separation occurred. In the remaining cases, the graft constricted from the base of the stock and developed a foot after it had become a distinct individual.

Experiment 3. On April 7, the head only of a six-tentacled polyp was grafted on a seven-tentacled hydra below the middle region (Fig. 18). A constriction between the graft and stock was noticed on April 9, and the graft separated from the stock on April 12, without having increased in length or having migrated any nearer to the aboral end of the stock. A like result was obtained in the four other similar grafts that were made.

The above experiments in lateral grafting seem to me to show conclusively that the axial relations assumed by the parts of the compound are important factors in determining not only the ultimate fate of the graft, but also the manner in which it will separate from the stock, if separation of the component parts is to occur. If the axes of the parts above the place of union of graft and stock come to form equal angles with the axis of the common trunk, the graft slowly migrates downward to the foot of the stock where constriction and final separation of the polyps takes place. If the axes of the parts above the line of union of graft and stock do not become equally inclined to the axis of the common trunk, then the graft seems able to exert its individuality more quickly than is the case with the other grafts, as it develops a foot on its aboral end and constricts off from the stock having undergone little or no downward migration towards the foot of the stock. If the axis of the graft swings around in line with that of the common trunk, then the graft is incorporated into the stock and the upper part of the stock becomes bent over where the graft is attached and moves downward and finally constricts off from its own aboral end. Whatever the fate of the graft, the final result in all of these lateral graft experiments is the formation of one or more normal individuals. I can agree fully with the statement of RAND that »No lateral grafts in H. viridis will persist as permanent abnormalities«.

2. Lateral Grafting to Produce Heteromorphosis.

Previous experiments made by WETZEL (10), PEEBLES and myself have shown that it is possible to produce heteromorphosis in hydra by means of end-to-end grafting. The following experiments were made to ascertain if heteromorphosis can also be produced by means of lateral grafting.

Experiment 1. In a preliminary set of eight experiments made on Nov. 18, 1901, the head of a hydra was removed by a transverse cut just behind the circle of tentacles, and the oral end of the polyp was then inserted into the side of another polyp as nearly as possible in the middle region as shown in Fig. 19. The fate of the graft was very similar in each experiment. A few hours after union was effected, the compound was usually found to be attached not only by the foot of the stock, but also by the aboral end of the graft which had bent over until its aboral end came in contact with the bottom of the dish. The compound then either formed a ʌ shaped structure, or it appeared as in Fig. 20, the graft and the aboral portion of the stock extending out in opposite directions while the head of the stock projected upward from the middle region of the compound. In every case the components of the graft seemed to be making a constant effort to pull away from each other. On Dec. 2, the graft appeared to be much nearer the foot of the stock in two of the compounds than when the operation was made and the portion of the trunk of stock above the line of insertion of the graft was correspondingly increased in length. A few days later, the same downward migration of the graft was perceptible in all the other six compounds. Each compound was attached by its two aboral ends and a constriction had appeared between the graft and stock by Dec. 8 (Fig. 21). Three of the grafts constricted from the stock on Dec. 20, without having migrated any nearer the foot of the stock than shown in Fig. 21. By Jan. 10, 1902, the remaining five grafts had constricted off from the stock in the same way. After each graft had separated from the stock it quickly developed a hypostome and tentacles at its oral end and became a normal individual.

Experiment 2. On Nov. 19, ten grafts were made as in experiment 1 and the following day the foot of each graft was removed by a transverse cut (Fig. 19, *EF*), in the hope that a head would develop on the cut aboral surface of the graft. In the course of 48 hours a foot formed on the cut surface in every case. The foot of each graft was then removed a second time, leaving but a small piece of the graft attached to the stock. In four cases a third foot formed on the cut surface and the graft subsequently separated from the stock as in the first experiment. In the remaining six cases, no regeneration took place from the cut surface. A gradual process of absorption of the graft by the stock began in the course of a few days, resulting in the formation of an apparently normal individual from the complete union of the two components.

The results obtained in the preceding experiments are very similar to those which MARSHALL (3) and I observed in experimenting with double-footed hydras produced by splitting a polyp longitudinally through the aboral end. Such abnormal polyps are always attached by both foot-ends, and each piece seems constantly endeavoring to separate from the rest of the polyp. In the course of a few days or weeks, depending entirely on the extent of the longitudinal cut in the beginning of the experiment, one aboral piece, invariably the smaller if the cut did not pass exactly through the middle of the polyp, constricts off from the rest of the hydra. It soon develops a hypostome and tentacles at its oral end and becomes a normal individual. The entire process of constriction and subsequent regeneration of these aboral ends of a double-footed polyp seems to be exactly similar to that taking place in the first experiment of the above series of lateral grafts. Moreover, if the aboral end of one part of a double-footed polyp is removed by a transverse cut, a new foot forms at once on the cut surface if the piece remaining is of considerable size, if, however, by removing the foot, only a small piece remains attached to the main part of the hydra, there is no regeneration at all from the cut surface. The small piece is gradually absorbed by the polyp until a normal form is again produced, a result similar to that obtained in six of the lateral grafts made in preceding series of experiments.

Experiment 3. Five grafts were made as in experiment 1. The aboral end of each graft polyp was then removed (Fig. 19, *EF*), and, subsequently, a longitudinal cut was made through the middle of the graft and extending transversely across the stock also (Fig. 19, *CD*). By this operation a long, narrow piece of the graft remained attached to the anterior part of the stock hydra, and a similar piece of the graft was united to the posterior part of the stock hydra. The object of this experiment was to ascertain if it would be possible to obtain a regeneration of a head on the aboral surface of the graft by greatly increasing the amount of cut surface. The history of four of the anterior pieces was as follows: A foot developed on the aboral surface of the graft two days after the operation and the compound became attached by it (Fig. 22, *F*), while the aboral end of the stock healed over but did not regenerate. In the course of a week the graft swung around in line with the axis of the stock, and four weeks after the experiment was begun a normal polyp was formed from the compound in all four cases. In the remaining case, the graft constricted from the stock a few days after the operation. It developed a hypostome and tentacles on its oral end and soon became a complete individual.

In three of the posterior pieces of the stock to each of which a narrow strip of the graft was attached, the following result was obtained.

A head developed on the anterior end of the stock in about three days after the operation and the graft appeared as a small protuberance on the side of the stock (Fig. 23). The graft gradually decreased in size and was finally completely absorbed. In one of these experiments, polyps of decidedly different shades of green had been grafted together, and in this case the new head that regenerated on the anterior end of the stock was found to be composed entirely of the tissues of the stock, the graft taking no part whatever in its formation.

The piece of graft remaining attached to the posterior portion of the stock was considerably larger in two cases than it was in the three compounds just described. A foot regenerated on the aboral surface of the graft soon after the operation, and the graft bent down so that its foot became attached. A new head developed from the oral end of the stock as in the previous experiments, and, as before, this new head was composed entirely of the tissues of the stock polyp. The appearance of the compound a week after the operation was much like that in Fig. 20. The greater part of the compound had assumed a horizontal position and the head of the stock extended up from the middle of the structure. In one case, separation of the graft from the stock took place on Dec. 30, and in the other case on Jan. 6. In neither of these compounds did the graft move below the middle of the stock before separation was effected.

Experiment 4. On April 12, the posterior half of a polyp was grafted into the side of another polyp by its oral end (Fig. 24). The day following the operation, a transverse cut was made across the graft hydra at a short distance from its place of attachment to the stock (Fig. 24, *AB*), thus exposing a freshly cut aboral surface on the graft polyp. This experiment was made in order to see if the axis of the small piece of graft would swing around in line with that of the common trunk and a head be regenerated on its aboral surface — the anterior part of the stock hydra subsequently moving down to its own foot end and constricting off as was the case in the experiment described on page 216.

The injured surface of the graft healed over in the course of a few hours, but no subsequent regeneration took place from it. The axis of the stock remained straight and the graft appeared as a small projection on the trunk of the stock. By the gradual absorption of the graft by the stock a normal polyp was formed from the compound some three weeks after the operation.

The above experiment was repeated eight times and in each case the graft was absorbed by the stock and a normal individual formed.

Experiment 5. On Jan. 13, six grafts were made in the following manner: The foot of a polyp was removed by a transverse cut and the aboral end of the trunk was then inserted into the middle region of another polyp (Fig. 25). About three hours after the operation a cut was made across the stock just below its line of union with the graft (Fig. 25, *ZY*). The next morning, when the cut surface *ZY* had healed over, the head of the graft was removed by a transverse cut behind the circle of tentacles (Fig. 25, *AC*), as it was not considered advisable to make both cuts *ZY* and *AC* at the same time on account of the severity of such an operation. It was hoped, when the experiment was made, that the graft would swing around in line with the stock and that a normal polyp be produced by the regeneration of a foot in the cut oral surface *AC*.

In four of the experiments the following results were obtained. Three days after the operation a slight projection was noticed on the aboral end of the stock, and tentacles were beginning to bud out on the oral end of the graft (Fig. 26). Until this time the axis of the graft had remained nearly at right angles to that of the stock, but now a change in the axial relations of the parts of the compound took place, so that both heads were nearly upright and their axes formed equal angles with the axis of the common trunk (Fig. 27), the compound being attached by a foot that had developed from the projection at the aboral end of the stock. A gradual constriction of the graft from the stock took place as in Fig. 6, and the two polyps finally separated three weeks after the operation.

The fate of the grafts in the other two experiments in this set was very different from that of the grafts described above, owing possibly to the fact that somewhat more than the head of the graft was removed by the cut *AC*. A few days after the operation, a slight projection appeared at the aboral end of the stock as in the other compounds of the set, but it did not develop into a foot, neither did a hypostome and tentacles regenerate from the oral surface of the graft. On Jan. 19, each compound appeared as in Fig. 28. By Jan. 24, the projection at the aboral end of the stock had almost disappeared, and the compound was attached by a foot which had developed on the oral end of the graft, while the graft itself was gradually coming in line with the axis of the stock (Fig. 29). A month after the operation each compound formed a perfectly normal hydra.

Besides furnishing new cases of heteromorphosis, these last experiments give additional evidence that the size of the graft as well as the axial relation which it bears to the axis of the stock, are of great importance in determining its ultimate fate.

Experiment 6. After the removal of the head of a dark green polyp, the oral end of the trunk was grafted into the side of a light green polyp near the middle region (Fig. 30). A few hours later the anterior end of the stock was cut off just above the line of union with the graft (Fig. 30, *ZY*), and the following day the foot of the graft was also removed by a transverse cut (Fig. 30, *AC*). It was hoped that the graft would swing around in line with the body of the stock and develop a head on its cut aboral surface *AC*.

In four of the six experiments that were made the fate of the graft was the same. A new head grew out from the cut oral surface *ZY*. It was composed entirely of the light colored tissue of the stock, the tissues of the graft taking no part whatever in its formation. Although the cut surface of the graft healed over at once, no regeneration took place from it, and the graft appeared as a small projection on the side of the stock (Fig. 31). The graft was gradually absorbed and a normal individual produced from the compound.

In one experiment made on Jan. 11, a foot regenerated on the aboral end of the graft and a head developed on the oral end of the stock in three days after the operation. The graft soon bent down so that its foot became attached, and the compound, therefore, appeared as a double-footed hydra (Fig. 32). On Jan. 26, the graft had migrated to below the middle region of the stock and it had decreased considerably in size (Fig. 33), thus indicating that it was undergoing a gradual process of absorption although it was moving towards the aboral end of the stock as if it were eventually to separate as a distinct individual. On Feb. 4, the compound appeared as in Fig. 34. The graft, which was much less than half of its former size, had reached the base of the stock, and the compound was seemingly attached by a very broad, flat foot, slightly indented in the middle line. By Feb. 17, the graft had become entirely absorbed and a perfectly normal polyp was formed from the compound.

It seems probable the behavior of this compound can best be explained as due to the size of the grafted piece. The graft was evidently too large to be absorbed at once, as were the grafts in the first four experiments in this set, and yet was not large enough to exert its individuality sufficiently to separate from the stock although it migrated to the foot of the stock before it was finally absorbed by the larger components.

In the last experiment in this series, two heads regenerated from the anterior end of the compound. One was composed entirely of the light colored tissue of the stock and had five tentacles, while the other head, regenerated on the graft, was dark in color and had but four tentacles. A foot developed on the aboral surface of the graft, and the compound soon became attached by two aboral ends (Fig. 35). During the following week, the two heads grew larger and each developed another tentacle. Two weeks after the operation the polyps had so oriented themselves that the whole structure appeared as a cross, the two heads forming the horizontal arms, while the compound was attached at the ends of the vertical arms Fig. 36). A week later, when all distinction in color between the polyps had disappeared, the two components were connected only by a narrow band of substance in which, for a time, both body layers were present (Fig. 37). This connection gradually became thinner, and for several days the polyps were united by a mere thread of ectoderm which finally broke so that the two polyps were completely separated.

It seems probable that in this case the cut *ZY*, Fig. 30, which removed the anterior end of the stock hydra also accidentally took off a small piece of the oral end of the graft. As a result, a freshly cut surface was formed on the oral end of the graft, and, therefore, a head regenerated on the cut surface just as if the polyp was not united to another individual.

3. Experiments to Determine the Effect of Lateral Grafting on the Regeneration of Tentacles.

Rand (8) found, in the course of his experiments, that after the removal of the head of a polyp by a transverse cut just behind the circle of tentacles, the number of tentacles that will regenerate is, with very few exceptions, less than the number removed in the beginning of the experiment. If the size of the polyp is still further reduced by removing the foot as well as the head, then still fewer tentacles will regenerate. From these experiments Rand concluded that »in pieces destitute of tentacles, the number produced varies with the size of the piece«. As the size of the hydra is, therefore, of importance in determining the number of tentacles that will regenerate in a given case, will it be possible to increase the number of tentacles by grafting the trunk of hydra on another polyp and thus, for a time at least, greatly increasing the volume of the polyp and the source from which it can obtain its supply of nourishment?

After the removal of the foot of a polyp, the aboral end of the trunk was inserted into the side of another polyp in the middle region (Fig. 8). The next day, after having noted the number of tentacles, the head of the graft was removed by a transverse cut Fig. 8, *A B*). The experiments grouped under this series were made at six different times, and upon polyps taken from different aquaria or brought from a pond near the laboratory. In every case the number of tentacles that had regenerated was counted ten days after the operation, as previous experiments had shown that new tentacles seldom appear after this length of time. A summary of the various experiments in this series is given in the following table.

Table VI.

	No. Hydras	Original No. of Tentacles	No. Hydras	No. of Tentacles regenerated
	9	8	1	9
	20	7	3	8
	9	6	7	7
	2	5	21	6
			6	5
			2	4
Total	40	276	40	246
Average		6.90		6.15

In the above table it is seen that only in one instance did more tentacles regenerate on the head of a graft than had been cut off in any case, and that the greater number of polyps regenerated six tentacles, although one half of the individuals had originally 7 tentacles.

Rand cut off the heads of 52 hydras and found that, whereas the average number of tentacles originally possessed by the polyps was 6.9, the average number of tentacles that regenerated was but 6.1, a difference of 0.8. In repeating Rand's experiments I found that in a total of 52 individuals with an average of 7.23 tentacles per hydra before the experiment began, the average number of tentacles that regenerated was 6.35 per hydra, a difference of 0.88. These results obtained on single polyps are practically the same as in the above experiments on lateral grafting where the average difference between the original number of tentacles on the head of the graft and the number regenerated ten days after the operation was 0.75.

Comparing the results obtained by Rand and by myself on single polyps with those summarized in the above table, it is evident that grafting one polyp on to another polyp does not cause a marked increase in the number of tentacles that will regenerate after the removal of the head of the graft. The graft, which sooner or later is destined to separate from the stock, regenerates exactly as it would have done had it not been united to another polyp. It is only when the graft is too small to exert its own individuality that its subsequent fate is influenced by the other component of the graft.

Owing to an accident, 18 of the 40 compounds in this series were killed about two weeks after the experiment began, so that the manner of the separation of the graft from the stock was not determined. Of the remaining 22 compounds, 16 developed a foot on the aboral end of the graft, and the two components separated in from 2—3 weeks, no perceptible migration of the graft towards the foot of the stock having taken place. In the other 6 compounds, there was a slow downward movement of the graft to the aboral end of the stock where for several days before final separation the two polyps were connected by only a narrow band of substance as in Fig. 6.

A number of experiments were made in which, after the removal of the foot, one-half or less than one-half of the posterior part of a polyp was grafted by its aboral end into the middle of another hydra, in order to study the relation between the number of tentacles that would regenerate and the original number possessed by the graft hydra. In the great majority of cases the graft was slowly absorbed by the stock, and, therefore, no definite conclusions could be drawn from this set of experiments. It was noted, however, in the few cases where a new head regenerated on the graft, that the number of tentacles was considerably less than the number of tentacles possessed by the graft polyp at the beginning of the experiment.

B. Grafting to Determine the Influence of the Larger on the Smaller Piece.

In one of the experiments made by Wetzel (10), the foot ends of two Hydra grisea were removed by transverse cuts, and the two aboral surfaces of the polyps were then grafted together. Subsequently the head of one component of the graft was cut off just back of the circle of tentacles. A few days later, a foot developed on the cut oral surface and a normal hydra was formed by a permanent union of the two components of the graft. This is the first recorded case of heteromorphosis in Hydra. Wetzel's experiment was repeated by Peebles (7); but she was not able to obtain a case of heteromorphosis, although she made the experiment 15 times. Peebles then grafted together the oral ends of two polyps after the removal of the hypostome and tentacles, and soon after union was completed one component was cut off close to the line of union. A new head developed on the cut, aboral surface of the smaller component in 5 of the 22 grafts that were made.

In my previous experiments on grafting hydra I removed the foot from a light and also from a dark green polyp and then united the polyps by their aboral surfaces. After union was completed, I cut off each component of the graft by a transverse cut close to and at equal distances from the line of union, thus forming a small ring of tissue composed of two components of nearly equal size and having two exposed oral surfaces. In every case a normal polyp was formed by the development of a foot on one oral surface and the regeneration of a head on the other. The same result followed when the heads of two polyps were removed and the oral ends grafted together, each component being subsequently cut off close to and at equal distances from the line of union. In the few cases where the cuts were made some distance from the line of union, each cut surface regenerated a structure like that removed and the two polyps eventually separated as complete individuals.

In grafting experiments such as those described above the question arises, what is the factor that determines on which component the head will regenerate, and on which the foot? Is it merely through chance that in some experiments heteromorphic structures develop, or does the larger component influence the smaller component in such a way that the latter's polarity is reserved, if necessary, in order that a normal polyp may be formed from the compound? The following experiments were made with the idea of determining, the answer to this question. In every experiment in this group, individuals of decidedly different colors were grafted together, as only by this means could it be accurately determined what part each component played in the subsequent regeneration.

Experiment 1. On April 10, the foot ends of two hydras were removed and the polyps were then grafted together by their aboral surfaces (Fig. 38). After the parts were firmly united, a transverse cut was made through the dark colored polyp quite close to the line of union of the two components (Fig. 38, *A B*). The anterior half of the light colored component was also removed by a transverse cut (Fig. 38, *E F*). These cuts produced a compound composed of a small ring of dark colored tissue, and a somewhat larger ring of light colored tissue, each having an exposed oral surface. On which of the components will a heteromorphic foot develop?

The above experiment was repeated 9 times with a like result in each case. A new head always regenerated on the oral surface of the larger component and a foot developed on the oral surface of the small ring of dark colored tissue, so that a normal polyp was formed by a permanent union of the two components of the graft.

Experiment 2. In the reverse of the above experiment, the oral ends of two polyps were united together after the removal of the hypostome and tentacles. Transverse cuts were then made as in Fig. 39, *A B*, *C D*, resulting in the formation of a compound with two exposed aboral surfaces. The lighter colored component was the larger as in the preceding experiment. Again the polarity of the larger component determined the kind of regeneration from the exposed surface of the smaller component, as in all but one of the 7 experiments that were made, a foot developed on the cut aboral surface of the larger component and a head on the exposed aboral surface of the smaller component, thus producing a normal individual.

In the one exceptional case in this group of experiments, a foot developed at each end of the compound, and one head with 5 tentacles regenerated at the line of union of the two components. The new head was formed entirely of the light colored tissue of the larger component (Fig. 40). During the following week, the larger component increased somewhat in length, and developed two more tentacles. A gradual downward migration of the smaller component towards the aboral end of the larger component was soon noticed, and three weeks after the operation all distinction in color between the parts of the compound had disappeared. By this time the smaller component had nearly reached the aboral end of the larger component and it was about one-half of its former size, showing that it was being slowly absorbed by the larger piece (Fig. 41). Five weeks after the operation, the smaller component had been completely absorbed and a perfectly normal hydra was formed from the compound.

In this last experiment the dark colored component was somewhat larger than it was in the other experiments of the set, and, consequently, the larger piece was not able to cause a reversal of the polarity of the smaller piece within a few days after the operation, although it was able to prevent the separation of the smaller component and to incorporate it into its own body to form a perfect individual.

The above experiments show that the kind of regeneration from the cut surface of a component of an end-to-end graft is determined, to a certain extent at least, by the size of the components. If the components are of sufficient size, each will exert its own individuality and regenerate a new structure on its cut surface like that removed, no matter how large the other component may be. Eventually the two polyps will separate and become complete individuals. If one component is much smaller than the other, the larger component either brings about a reversal of the polarity of the smaller component thus causing the regeneration of a heteromorphic structure from the cut surface of the smaller component; or else the smaller component is completely absorbed by the larger. In any case the final result is the formation of one or two individuals of normal form.

C. Tangent Grafting.

Experiments made by **Peebles**, **Hefferan** and myself have shown that it is not possible to produce a permanent hydra body of twice the length of the normal polyp by grafting several pieces of hydra together in the same or in a reversed direction. In such grafts the parts adhere readily enough at first, and they remain united for some days, but eventually as many separate individuals will be formed as there are heads produced on the compound.

Will it be possible to obtain a permanent union of two hydras so that the diameter of the body shall be doubled, but the length not increased? The following experiment was made a number of times to test this point, but in only two cases was it found possible to obtain anything like a satisfactory union of the tissues. A small, sharp scalpel was inserted in the mouth of a large polyp and the body wall was split down one side to the very end of the foot. The cut edges at once separated so that the hydra became, for a short time, a flat sheet of tissue. Another hydra, similarly cut, was quickly placed on the first polyp so that the endodermal layers were in contact. It was hoped that, when the cut edges of one polyp closed in, they would adhere to the cut edges of the other polyp and thus form a polyp of twice the usual diameter. Such a compound may, perhaps, be called a »tangent graft«, although **Hefferan** used this term to designate grafts »in which the polyps have had shavings or slices cut from the trunks and are put together side by side without removing either end«. The following experiments differ from those of **Hefferan** in that no part of the body wall was removed. In making the compound the polyps were placed together so that the head of one polyp came in contact with the foot of the other as in Fig. 42. None of the experiments in which the two oral ends of the polyps were placed in contact gave satisfactory results.

A description of one of the most interesting of these tangent grafts is as follows: A few hours after the operation the compound appeared as in Fig. 43, the two polyps, one with six, the other with seven tentacles, being joined together in two places only. At one end of the compound the polyps were firmly united for about one third of their length, and near the middle of the compound, the polyps were held together by a narrow band of substance. The aboral end of one polyp and the oral end of the other were completely free.

The operation was made on Nov. 21, and by Nov. 25, the compound appeared as in Fig. 44. The head of the hydra designated in the figure by the letter *B* had become firmly united with the aboral end of the hydra marked *A*. All connection between the head and the trunk of hydra *B* was broken so that the component parts were united only by the narrow band of substance in the middle region. On Nov. 30, a small projection was noticed on the aboral end of hydra *A* (Fig. 45, *O*), and it soon became a foot as it adhered to the needle when touched. From its location I judged that this foot was part of the original foot of hydra *A* and not an entirely new formation. By Dec. 2, the compound was found to be attached by a foot that had developed on the oral end of component *B* (Fig. 45, *Y*), and a bud was also forming on the side of this polyp. During the following week, the connection between polyp *B* and the rest of the compound grew more slender, and the axial relations of the components became such that component *B* supported the rest of the compound entirely, thus forming a T shaped structure (Fig. 45). On Dec. 9, *B* separated from the rest of the graft and disintegrated without any regeneration taking place. A constriction was soon noticed near the middle of the rest of the compound (Fig. 46) and this constriction deepened rapidly so that two normal individuals were formed on Dec. 27. One of the polyps received the foot that had appeared at the posterior end of component *A* (Fig. 45, *O*).

In the other partially successful graft in this series, the two polyps were united for a time by a broad band in the middle region only. In the course of a few days one polyp swung around so that the compound was attached by the two aboral ends of the components. At the end of three weeks the polyps had separated as distinct individuals. This result is similar to that obtained by HEFFERAN in some of her tangent grafts of Hydra fusca where the polyps were united in a reversed direction.

These experiments, together with those of PEEBLES and HEFFERAN seem to show that it is not possible to permanently change the form or size of a polyp by experimental means. Abnormalities will persist for a time, but sooner or later regulative processes occur which result in the formation of normal individuals.

III. The Regulation of Four-headed Hydras.

In my former experiments, double-headed polyps were formed by cutting a polyp longitudinally from the oral end to within a short distance of the foot. In such polyps regulation to a normal form was brought about by a gradual splitting of the trunk of the polyp until two individuals were formed from the one. The following experiments were made to see if regulation to a normal form would be the same in four-headed hydras as it is in double-headed hydras.

Two methods were employed to produce four-headed hydras, both of which proved equally successful. One method was to make two longitudinal cuts through the oral end of a large polyp and then, by means of needles, keep the parts from reuniting for some three hours after the operation (Fig. 47). Each of the four parts into which the anterior end of the polyp was cut closed in and formed a head. In the second method used, double-headed polyps were first made by splitting a hydra longitudinally through the middle line for some distance, and then, on the following day, each head was again split longitudinally thus producing a four-headed hydra.

The regulation of these four-headed polyps varied somewhat in the different individuals, although the end result was the same in all cases. A description of one of these experiments only will be given. The experiment was made on Nov. 1 by the first method described. The hydra experimented upon had orginally seven tentacles and there resulted from the operation an individual with four heads. Two of the heads had three tentacles each, one had one tentacle, while the remaining head was at first destitute of tentacles. On Nov. 5, the number of tentacles on each head had increased so that one head, *A*, had five tentacles; two heads, *B* and *D*, had four tentacles each; while head *C*, which did not receive any of the tentacles in the beginning of the experiment, had now regenerated three tentacles, thus making a total of 16 tentacles for the entire hydra (Fig. 48).

Heads *B* and *C* were formed from the middle part of the hypostome; and from the beginning of the experiment they were united for a short distance so that there were but three branches from the main trunk.

During the next three days, one more tentacle appeared on head *C* and one more also on head *D*, making a total of 18 tentacles for the whole polyp. On Nov. 13, head *D* was found to be much nearer the common foot end of the hydra, and, therefore, there must have been either a further splitting of the trunk of the polyp, or, possibly, a downward migration of head *D* towards the aboral end of its own trunk. At the same time, heads *B* and *C* were found to be undergoing a process of fusion as the hypostomes were much closer together than they were when observations were made on Nov. 8 (Fig. 49). On Nov. 17, the axial relations of the parts had changed so that the axis of head *D* was in a line with the axis of the aboral end of the common trunk (Fig. 50), and the axis of this part of the polyp formed nearly a right angle with the axis of the portion of the trunk supporting the other three heads. By Dec. 1, the fusion of heads *B* and *C* was completed, so that there was but one mouth opening surrounded by 9 tentacles (Fig. 51). Two days later a constriction was noticed between the part of the trunk bearing head *D* and the rest of the polyp. This constriction deepened rapidly and on Dec. 10, *D* separated as a distinct individual, leaving the two heads *A* and *BC* united for some distance at their aboral ends. During the following week, the portion of the common trunk uniting *A* and *BC* decreased considerably in length so that the polyps were united only at their foot ends by a narrow connection. On Dec. 20, the connection was broken and two normal individuals were produced.

In all ten of the experiments in this series, regulation to a normal form was brought about, as in the double-headed polyps previously studied, by a separation of the original hydra body into several pieces, each eventually becoming a normal polyp. It is only in rare instances that two heads fuse together as was the case in the experiment described above. Ordinarily there are as many separate polyps formed from the one as there are heads produced when the operation is first made.

IV. Experiments on Hydras Bearing Buds.

While the experiments described above were in progress, many of the stock hydras in the aquaria were budding freely. The following experiments were undertaken mainly to ascertain if it would be possible to bring about a complete absorption of the bud by the parent hydra, or to incorporate a portion of the parent hydra into the body of the bud.

Rand made several experiments in which he cut a budding hydra transversely just above and also just below the place of attachment of the bud. He thus obtained a bud united to a narrow ring of the body wall of the parent hydra, and in every instance, no matter how young the bud, the bud constricted off from the piece of the parent hydra in the course of a few days. In all of Rand's experiments, the ring of tissue from the body wall of the parent hydra which remained connected with the bud was of considerable size. The following experiment was made to see whether a bud will also constrict off from a very small fragment of the body wall of the parent hydra, or whether it will incorporate the piece into its own trunk, as is the case in grafting experiments where a very small piece of one polyp is grafted into another polyp of larger size.

Series I. In this set of 10 experiments, hydras bearing buds in various stages of development were operated on in the following manner: Two oblique cuts were made across the parent hydra (Fig. 52, *MN*), so that but a small triangular shaped piece of the body wall remained attached to the bud. The morning following the operation, the cut surfaces had healed so that a small, rounded piece of tissue was attached to the aboral end of the bud. In every instance the bud sooner or later constricted off from the fragment of the parent hydra, the time required for the separation depending entirely on the size of the bud when the operation was made. As far as could be determined, the process of separation of the bud from the small piece of the parent hydra was in no way different from that taking place under normal conditions, and the process was apparently not delayed. No matter, therefore, how small the piece of the body wall of the parent hydra attached to a bud, the bud will not absorb it, neither is the development of the bud affected by the operation.

Series II. In another set of 6 experiments, only hydras bearing very young buds in which the tentacles had not yet appeared were used. In these experiments, the parent hydra was cut transversely just above the place of attachment of the bud (Fig. 53, *MN*). The operation was made to see whether the bud would constrict off under these conditions, or whether it would remain attached to the posterior portion of the parent hydra and form the permanent head of the polyp. In every instance, on the day following the operation, the bud had developed tentacles and a slight constriction was noticed at its aboral end. The final separation of the bud from the piece of the parent hydra occurred in from 4—5 days after the operation in all 6 experiments.

Series III. In five cases, just after the tentacles had appeared, the head of a bud was cut off by a transverse cut just back of the circle of tentacles (Fig. 54) to ascertain if the separation of the bud from the parent hydra would be prevented or greatly delayed by the operation. A new hypostome and tentacles very quickly regenerated on the cut surface and the bud constricted off in from 3—5 days after the operation.

Rand succeeded in making a permanent union between a bud and the parent hydra by cutting through both bud and parent as in Fig. 55, *RS*. The half-bud remaining attached to the anterior part of the parent hydra constricted off in all five of the experiments that he made. In three cases, the half-bud attached to the posterior portion of the parent hydra swung around so that its axis was in line with that of the parent hydra and it became the permanent head of a normal polyp.

I repeated the above experiment of Rand's six times with the same result in each case, i. e. separation of the half-bud from the anterior portion of the parent hydra in the course of two or three days after the operation, and the swinging around of the half-bud attached to the posterior portion of the parent hydra to form the head of the polyp.

In a slight modification of the above experiment, a hydra bearing a very young bud was used. The head of the bud was removed by a transverse cut (Fig. 55, *TU*) and then a longitudinal cut was made through the body of the bud and extending across the parent hydra transversely as well (Fig. 55, *RS*). By removing the oral end of the bud, it was hoped the separation of the portion of the bud attached to the anterior part of the parent hydra would be prevented and that a foot would regenerate on the oral end of the bud (Fig. 55, *TU*).

The history of the anterior piece of the parent hydra and the fragment of the bud attached to it was as follows: On Dec. 6, the day following the operation, the cut surfaces had healed over and the bud projected out at right angles to the trunk of the parent hydra (Fig. 56). By Dec. 8, a foot had developed at the aboral end of the parent hydra and tentacles had appeared around the oral end of the bud which had increased considerably in size and was beginning to show a constriction at its base. The bud separated from the parent hydra on Dec. 12, seven days after the operation was made, separation being somewhat delayed, but not prevented by the operation. The same result was obtained in five other similar experiments. It appears impossible to effect a permanent union between the anterior portion of a hydra and the whole or part of one of its buds.

The fate of the piece of bud attached to the posterior portion of the parent hydra was alike in all six cases. The cut surface healed over in the course of a few hours, and two days later the bud and the parent hydra had each developed a new hypostome and tentacles at its oral end, while a slight constriction had appeared at the base of the bud (Fig. 57). Separation of the bud from the parent occurred, as in the anterior pieces, about seven days after the operation.

The above experiments show that while, in exceptional cases, it is possible to cause a permanent union between a parent hydra and a portion of a bud, this union occurs only when the axis of the bud swings around in line with the axis of the parent hydra, a condition which is always necessary in order that a permanent union may be effected between the components of a lateral graft. In the first experiments in this group, the fragment of the parent hydra remaining attached to the bud was much smaller than the graft hydra in many of the experiments in lateral grafting in which the graft became permanently incorporated into the body of the stock hydra; yet a bud is seemingly not able to absorb a part of the parent hydra and always separates from it sooner or later.

Summary.

1) The number of tentacles that will regenerate after the removal of the head of Hydra viridis depends, to a certain extent at least, on the presence or absence of light. If the polyps are kept in continued darkness during the time they are regenerating, fewer tentacles will be developed than is the case when the polyps are exposed to the influence of light. The rate at which the tentacles develop, however, is the same in both cases.

2) In lateral grafting experiments in which the aboral end of one polyp is inserted in the side of another polyp (Figs. 1, 8 etc.) the axial relations assumed by the components of the graft is an important factor in determining the fate of the graft and also the manner in

which it will separate from the stock in those cases where the union of the components is not a permanent one.

A) If the axes of the parts above the place of union of the two components of the graft form equal angles with the axis of the common trunk, the graft slowly migrates to the foot of the stock, and constriction and final separation takes place in this region (Figs. 3—6).

B) If the axis of the stock does not become bent at the place of insertion of the graft, the graft develops a foot on its aboral end (Figs. 8—10) and soon separates from the stock, having undergone no apparent downward migration towards the aboral end of the stock.

C) If the axis of the graft swings around in line with that of the common trunk, the graft unites permanently with the stock, and a part of the stock constricts off from its own trunk and becomes a separate individual (Figs. 11—14).

3) The downward migration of a graft towards the aboral end of the stock is not due to gravity or to a splitting of the stock. It is a movement of the graft alone and does not include a movement of some of the tissues of the stock also.

4) The fate of a graft does not depend upon its degree of specialization, but upon its size, upon the axial relations it assumes with respect to the stock, and upon its position in the stock. If the head only of a polyp is inserted into another polyp close to its oral end (Fig. 7), the grafted head fuses completely with the head of the stock and the abnormally large number of tentacles is reduced by fusion and absorption to within the limits of normal variation. If a large piece of one polyp is grafted anywhere along the trunk of another polyp, it will sooner or later separate from the stock and become a distinct individual (Figs. 8—10). A small piece of any part of the body wall of a polyp, if grafted on any part of another polyp, will be absorbed by the stock; but the head of a polyp can be incorporated only into the oral end of the stock.

5) If, after removing the head of a polyp, the oral end of the trunk is grafted into the middle of another polyp (Fig. 19), the graft will migrate towards the aboral end of the stock (Fig. 20), constrict off and become a complete individual by developing a hypostome and tentacles at its oral end. If the foot of the graft of such a compound is removed by a transverse cut (Fig. 19, *EF*), a new foot always regenerates, and the graft eventually separates from the stock. If the cut has removed all but a small piece of the graft, gradual

absorption of the graft by the stock takes place and a normal polyp is formed.

6) If, after the removal of the hypostome and tentacles, the oral end of a polyp is grafted into the middle of another polyp and then the foot of the graft removed by a transverse cut, and if, subsequently, a longitudinal cut is made through the graft and extending transversely across the stock (Fig. 19, *CD*, *EF*), the stock is divided into an anterior and a posterior piece to each of which a long, narrow piece of the graft is attached. If the part of the graft attached to the anterior portion of the stock swings around so that its axis is in line with that of the stock, a permanent union is effected between the graft and the stock, the graft regenerating a foot and becoming the aboral end of a normal polyp (Fig. 22). If the graft axis remains at nearly right angles to that of the stock, then the graft finally constricts off from the stock as a separate individual. If the piece of graft attached to the posterior portion of the stock is of sufficient size, it always separates from the stock; if it is small it is incorporated into the body of the stock (Fig. 23).

7) If the foot of a polyp is removed, and the polyp grafted by its aboral end into the middle of another polyp and if, subsequently, the head of the graft and the part of the stock below the line of union with the graft are removed by transverse cuts (Fig. 25) the fate of the graft depends on its size and the axial relations it assumes with respect to the stock. If the graft is large and its axis remains at nearly right angles to the axis of the stock, it develops tentacles on its oral end and eventually separates from the stock (Figs. 26, 27); if the graft is small, it swings around in line with the stock (Figs. 28, 29), develops a foot on its oral end and becomes the permanent aboral end of the polyp.

8) If the head of a polyp is removed and the oral end of the trunk grafted into the middle of another polyp, and then the foot of the graft and also the part of the stock above the place of insertion of the graft are removed by transverse cuts (Fig. 30), the fate of the graft depends on its size. If the graft is large it regenerates a foot on its cut aboral surface and eventually separates from the stock. If the graft is small, it is absorbed by the stock.

9) The number of tentacles that will regenerate after the removal of the head of a polyp, is not increased by grafting the polyp into another polyp, and thus, for a time at least, increasing the volume of the polyp.

10) The kind of regeneration from the cut surface of a component of an end-to-end graft is determined, to a certain extent at least, by the size of the component. If both components of the graft are of considerable size, each will regenerate a new structure like that removed and eventually the two components will separate as distinct individuals. If one component is much smaller than the other, it is either absorbed by the larger component, or it forms a permanent union with it. In the latter case its polarity is reserved, if necessary, in order that a structure may regenerate on its cut surface that will produce a normal polyp.

11) It is not possible to permanently increase the diameter of the trunk of a hydra by means of tangent grafting. Such abnormalities persist for a time, but sooner or later regulative changes occur which result in the formation of one or more normal individuals (Figs. 42—46).

12) Regulation in four-headed hydras is brought about, as in double-headed polyps, by a separation of the body of the hydra into distinct individuals. Ordinarily there are as many individuals produced from the one as there are heads formed in the beginning of the experiment; but in rare cases two heads may fuse together and a single individual be formed from this part of the polyp (Figs. 48—51).

13) If only a small, triangular piece of the body wall of a parent hydra remains attached to a bud in any stage of development (Fig. 52), the bud eventually constricts off from it. In no case is the piece of the parent hydra incorporated into the body of the bud.

14) It is possible to delay, but not to prevent the separation of a bud from the parent hydra by removing the anterior end of the bud just after the tentacles have appeared (Fig. 54).

15) By cutting longitudinally through the middle of a bud in which the tentacles are just forming and extending the cut across the parent hydra also (Fig. 55, *RS*), it is possible to bring about a permanent union between the posterior part of the parent hydra and the part of the bud attached to it in cases in which the bud swings around so that its axis is in a line with that of the parent hydra, as first determined by RAND. It is, however, seemingly impossible to bring about a permanent union between the anterior part of the parent hydra and the portion of the bud attached to it (Fig. 56).

Bryn Mawr College, Bryn Mawr, Pa., Nov. 8, 1902.

Zusammenfassung.

1) Die Anzahl der sich regenerirenden Tentakel nach Wegnahme eines Kopfendes von Hydra viridis hängt, wenigstens bis zu einem gewissen Grade, vom Zutritt bezw. der Abwesenheit des Lichtes ab. Werden die Polypen während der ganzen Zeit ihrer Regeneration ununterbrochen im Dunkeln gehalten, so entwickeln sich weniger Tentakel, als es der Fall ist, wenn die Polypen dem Einfluss des Lichtes ausgesetzt werden. Immerhin ist der Entwickelungsgrad der Tentakel in beiden Fällen derselbe.

2) Bei Versuchen mit seitlichen Vereinigungen, bei denen das aborale Ende des einen Polypen der Seite eines anderen eingefügt wurde (Fig. 1, 8 etc.), sind die Beziehungen der Achsen beider Komponenten ein wichtiger Faktor für das Schicksal des aufgepfropften Stücks und für die Art und Weise seiner Trennung vom Stock in denjenigen Fällen, in denen die Vereinigung keine dauernde ist:

a) Bilden die Achsen der Theile oberhalb der Vereinigungsstelle der beiden Komponenten gleiche Winkel mit dem gemeinsamen Stamm, so wandert das aufgepfropfte Stück allmählich an den Fuß des Stocks, woselbst es zur Abschnürung und schließlich Trennung kommt (Fig. 3—6).

b) Wird die Stockachse an der Pfropfungsstelle nicht gebogen, dann bildet das aufgepfropfte Stück einen Fuß am aboralen Ende (Fig. 8—10), und trennt sich bald vom Stock, ohne anscheinend eine Abwärtswanderung nach dem Stockfußende unternommen zu haben.

c) Schwingt sich die Achse des aufgepfropften Stücks in gleicher Richtung mit der Stockachse herum, so vereinigt sich das aufgepfropfte Stück dauernd mit dem Stock und ein Theil des letzteren schnürt sich von seinem eigenen Stamme ab und wird zu einem selbständigen Individuum (Fig. 11—14).

3) Die Niederwanderung eines aufgepfropften Stücks nach dem aboralen Stockende entsteht weder unter dem Einflusse der Schwerkraft noch durch Spaltung des Stocks. Sie stellt lediglich eine Bewegung des Pfropfstücks allein dar und bedingt keinerlei Gewebsverschiebungen des Stocks selbst.

4) Das Schicksal eines Pfropfstücks hängt nicht von dem Grade seiner Specialisirung ab, sondern von seiner Größe, von der relativen Lage seiner Achse zu der des Stocks und von seiner Stellung an letzterem. Wird das Kopfende eines Polypen in der Nähe des oralen Endes eines anderen Polypen angefügt (Fig. 7), so verschmilzt das Pfropfstück vollständig mit dem Kopf des Stocks und die abnorm große Tentakelzahl wird durch Verschmelzungs- und Resorptionsvorgänge so weit reducirt, bis sie innerhalb der normalen Variationsbreite liegt. Wird ein großes Stück von einem Polypen irgendwo entlang dem Stamm eines anderen Polypen angefügt, so trennt es sich früher oder später als selbständiges Individuum von dem Stock (Fig. 8—10). Ein kleines Stück aus der Körperwand eines Polypen wird an jeder beliebigen Stelle eines anderen Polypen von dem Stock desselben resorbirt; ein Polypenkopfstück lässt sich aber lediglich dem oralen Ende des Stocks inkorporiren.

5) Fügt man das orale Ende eines des Kopfes beraubten Polypenstammes der Mitte eines anderen Polypen ein (Fig. 19), so wandert das Pfropfstück nach dem aboralen Stockende (Fig. 20), schnürt sich ab und wird zu einem vollständigen Individuum, indem es an seinem oralen Ende ein Hypostom und Tentakel

entwickelt. Entfernt man den Fuß des Pfropfstücks an einer solchen Vereinigung durch einen Querschnitt (Fig. 19 *EF*), so bildet sich stets ein neuer Fuß und das Pfropfstück löst sich eventuell vom Stock ab. Hat der Schnitt Alles bis auf ein kleines Stück des Pfropfstücks entfernt, so findet schrittweise Resorption des Pfropfstücks seitens des Stocks statt und es entsteht ein normaler Polyp.

6) Heftet man nach Entfernung von Hypostom und Tentakel das orale Ende eines Polypen der Mitte eines anderen Polypen ein und entfernt dann den Fuß des Pfropfstücks durch einen Querschnitt, dem ein Längsschnitt durch das Pfropfstück und quer durch den Stock (Fig. 18 *CD*, *EF*) sich erstreckend nachfolgt, so theilt sich letzterer in ein vorderes und ein hinteres Stück, deren jedem ein langes schmales Stück des Pfropfstücks anhaftet. Schwingt sich die Pfropfstückhälfte des vorderen Stocktheils so herum, dass seine Achse mit der des Stocks zusammenfällt, so wird die Vereinigung des Pfropfstücks mit dem Stock zu einer dauernden, indem das Pfropfstück einen Fußtheil regenerirt und das aborale Ende eines normalen Polypen wird (Fig. 22). Bleibt die Achse des Pfropfstücks unter fast rechtem Winkel zur Achse des Stocks stehen, so schnürt es sich schließlich als selbständiges Individuum von ihm ab. Besitzt ein dem hinteren Stocktheil angeheftetes Pfropfstück genügende Größe, so trennt es sich stets ab; ist es klein, so wird es dem Stockkörper einverleibt (Fig. 23).

7) Entfernt man den Fuß eines Polypen und heftet ihn mit seinem Hinterende einem anderen in der Mitte an, wird dann nachträglich der Kopf des Pfropfstücks und der unterhalb der Vereinigungsstelle gelegene Theil des Stocks durch einen Querschnitt entfernt (Fig. 25), so hängt das Schicksal des Pfropfstücks von seiner Größe und der gegenseitigen Lage seiner und der Stockachse ab. Ist das Pfropfstück groß und bleibt seine Achse unter nahezu rechtem Winkel zu der des Stocks stehen, so entwickelt es Tentakel an seinem oralen Ende und trennt sich eventuell vom Stock (Fig. 26, 27); ist es aber klein, so stellt es sich in die Längsrichtung des Stocks ein (Fig. 28, 29), bildet einen Fuß an seinem oralen Ende und wird bleibend zum aboralen Ende des Polypen.

8) Wird der kopflose Stamm eines Polypen mit dem oralen Ende der Mitte eines anderen Polypen eingefügt, danach sowohl der Fuß des Pfropfstücks wie der oberhalb der Vereinigungsstelle liegende Theil des Trägers durch einen Querschnitt entfernt (Fig. 30), so hängt das Schicksal des Pfropfstücks gleichfalls von seiner Größe ab. Ist es groß, so regenerirt es einen Fuß an seiner aboralen Schnittfläche und trennt sich eventuell von seinem Trägerpolypen; ist es dagegen klein, so wird es von ihm resorbirt.

9) Die Tentakelzahl, die nach der Entfernung des Kopfendes von einem Polypen regenerirt wird, erfährt keine Vermehrung durch Aufpfropfung des Polypen auf einen anderen, wodurch doch, wenigstens eine Zeitlang, sein Volumen vergrößert wird.

10) Der Regenerationsvorgang an der Schnittfläche eines Komponenten einer endständigen Vereinigung ist wenigstens einigermaßen durch die Größe der Komponenten bestimmt. Sind sie beide groß, so regenerirt jeder die den verlorenen entsprechenden Theile und die beiden Theile trennen sich eventuell als selbständige Individuen. Ist ein Komponent viel kleiner als der andere, so wird er von letzterem entweder resorbirt oder er geht mit ihm eine bleibende Verbindung ein. Im letzteren Fall wird nöthigenfalls seine Polarität zu Gunsten der Erzeugung eines normalen Polypen derart umgekehrt, dass sich an der Schnittfläche die dementsprechende Neubildung entwickelt.

11) Eine bleibende Vergrößerung des Stammdurchmessers durch tangentiale Einpfropfung zu erreichen gelingt nicht. Solche Abnormitäten bleiben wohl eine Zeitlang bestehen, früher oder später treten aber Veränderungen ein, die zur Bildung eines oder mehrerer normalen Individuen führen (Fig. 42—46).

12) Die Regulation bei vierköpfigen Polypen von Hydra erfolgt wie bei zweiköpfigen dadurch, dass sich der Körper in getrennte Individuen theilt. Gewöhnlich entstehen so viele Individuen aus dem einen, als Köpfe im Anfang des Versuchs gebildet worden sind; in seltenen Fällen können aber auch zwei Köpfe verschmelzen und aus diesem Theil des Polypen nur ein Individuum entstehen (Fig. 48—51).

13) Wenn nur ein kleines dreieckiges Stück der Körperwand einer elterlichen Hydra an einer Knospe derselben, wie weit dieselbe immer entwickelt sei, hängen bleibt (Fig. 52), so schnürt sich die Knospe eventuell von ihm ab. Niemals wird das Stück des Elternthieres dem Körper der Knospe einverleibt.

14) Man kann die Trennung einer Knospe von der elterlichen Hydra verzögern, aber nicht verhindern, indem man das Kopfende der Knospe unmittelbar nach dem Auftreten der Tentakel entfernt (Fig. 54).

15) Schneidet man eine Knospe der Länge nach mitten durch und verlängert man den Schnitt noch durch das Elternthier hindurch (Fig. 55 *RS*), so ist es möglich, eine bleibende Verbindung zwischen dem Hintertheil des Elternthieres und dem an ihm gebliebenen Theil der Knospe herbeizuführen, nämlich dann, wenn die Knospe sich bis zum Zusammenfallen der beiderseitigen Achsen herumbiegt, wie schon RAND feststellte. Dagegen ist es anscheinend unmöglich, eine bleibende Vereinigung des Vorderendes des Elternthieres mit dem ihm anhängenden Knospentheile zu erzielen (Fig. 56).

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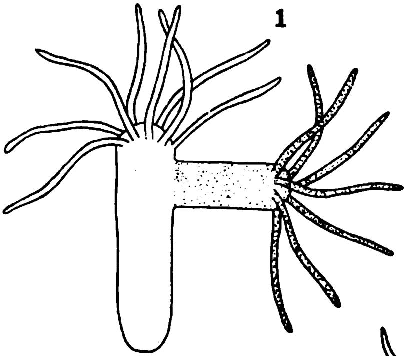

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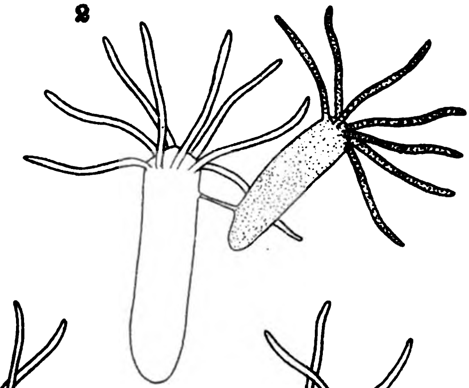

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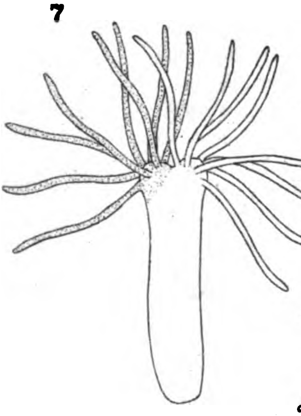

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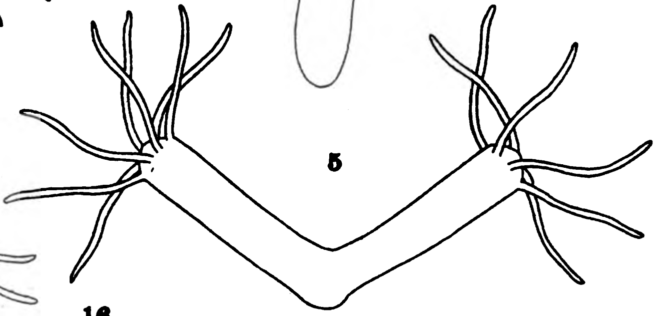

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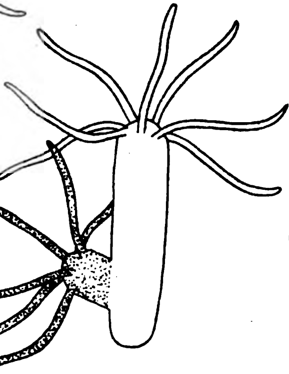

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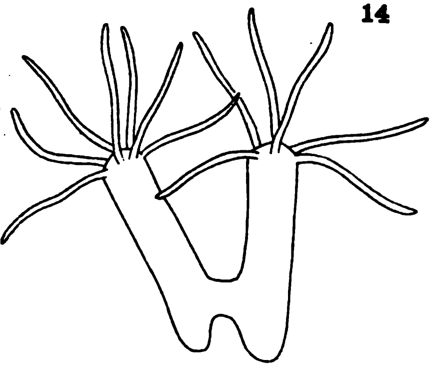

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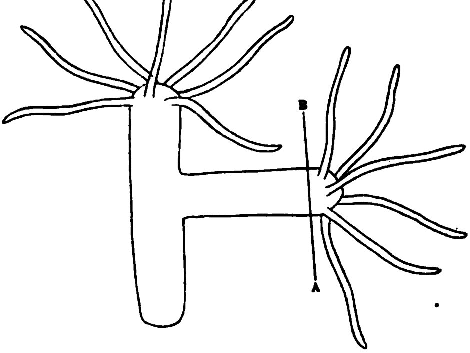

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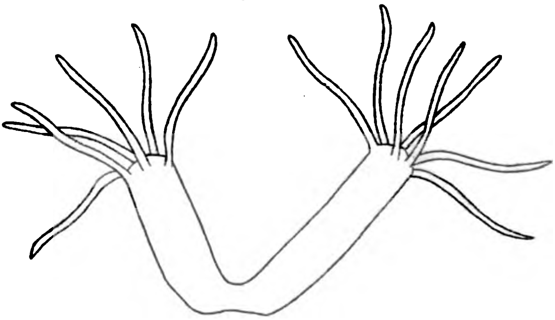

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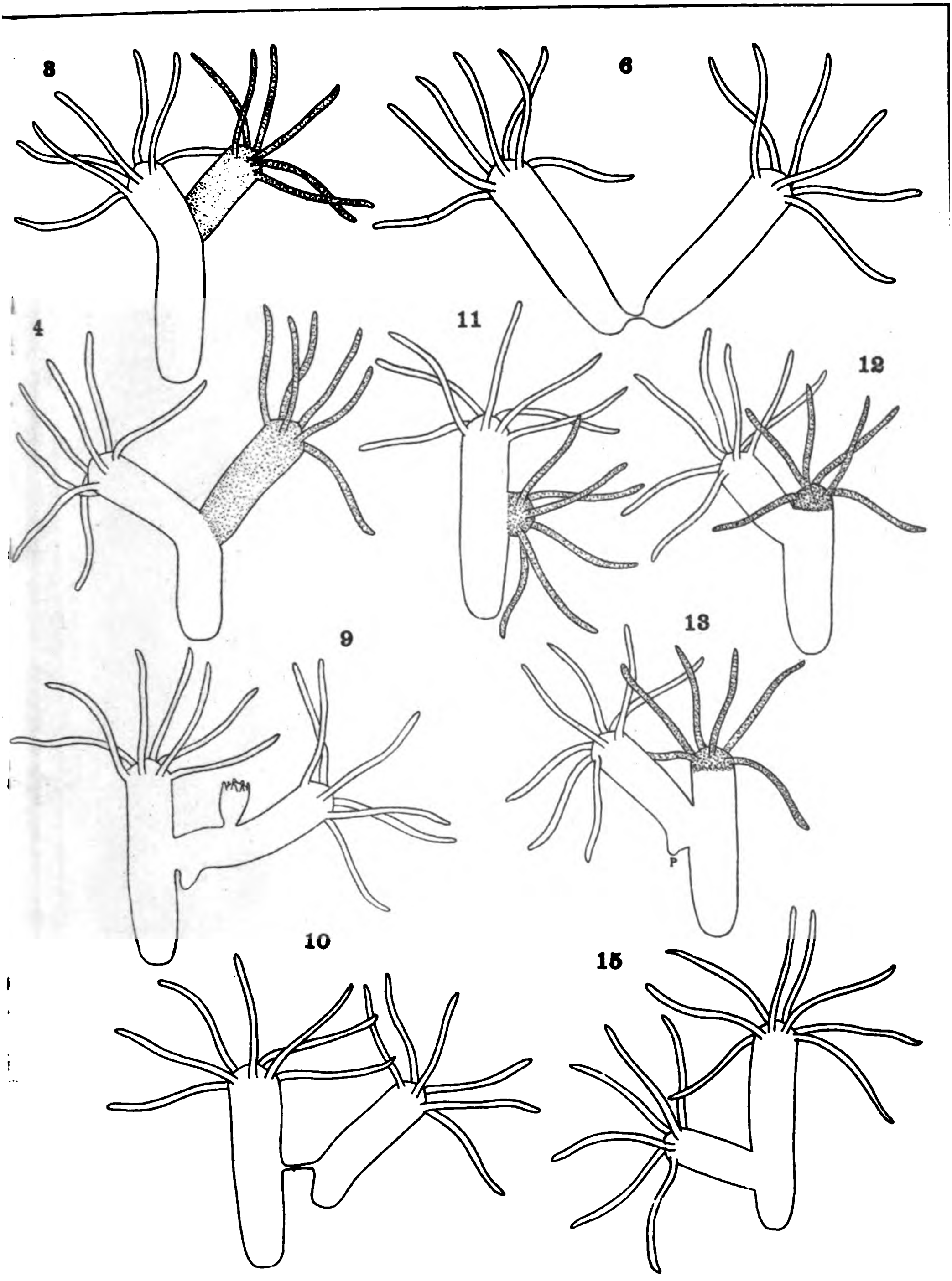

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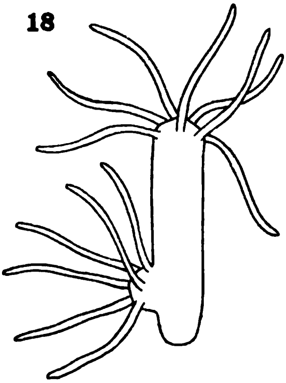

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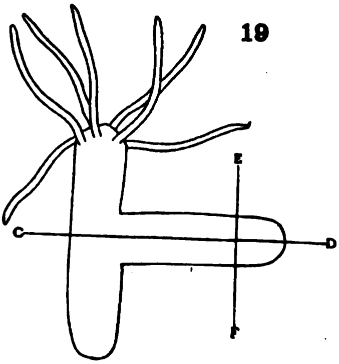

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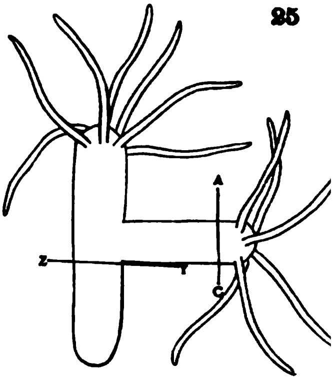

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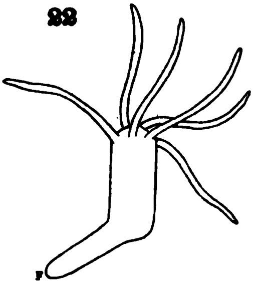

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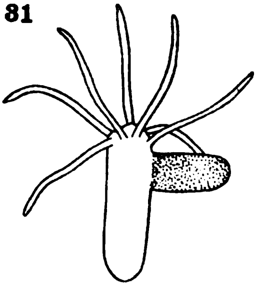

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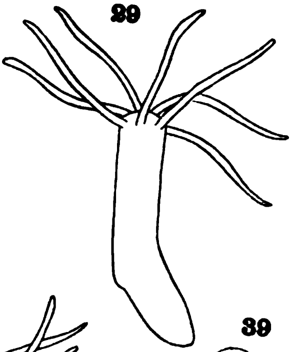

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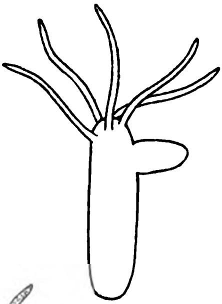

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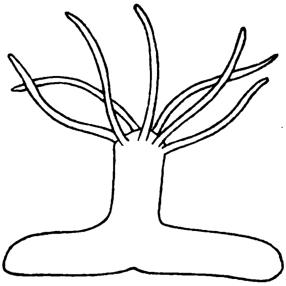

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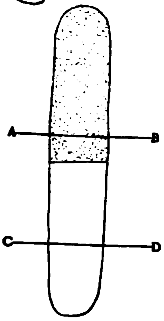

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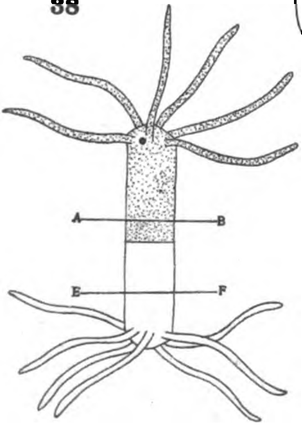

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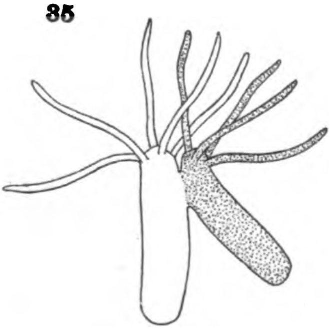

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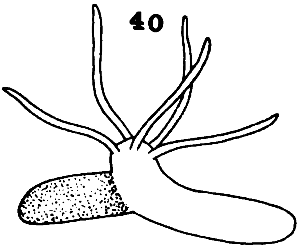

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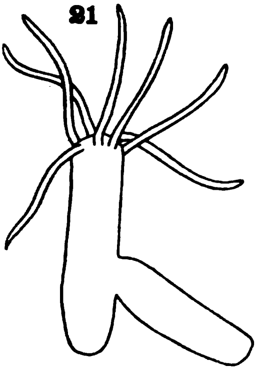

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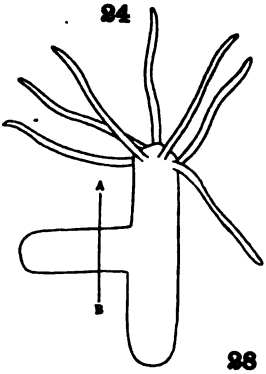

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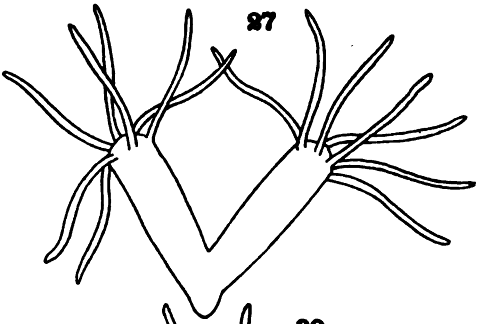

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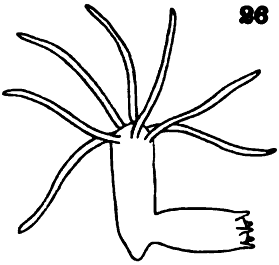

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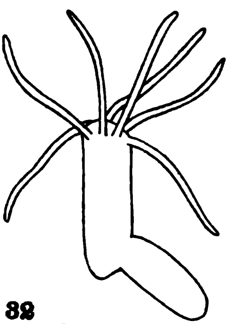

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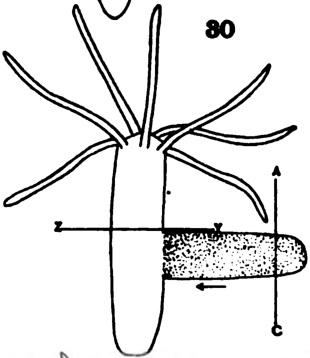

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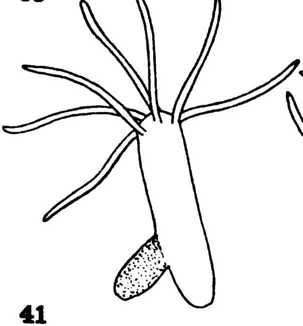

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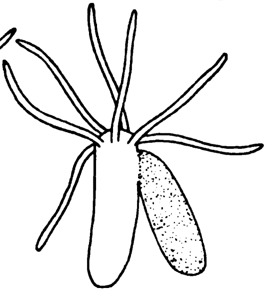

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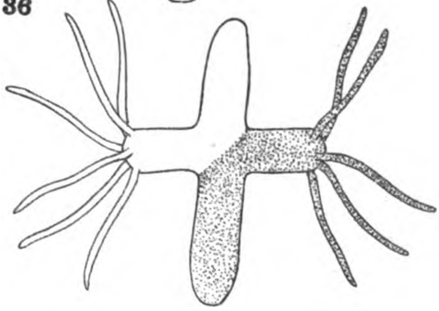

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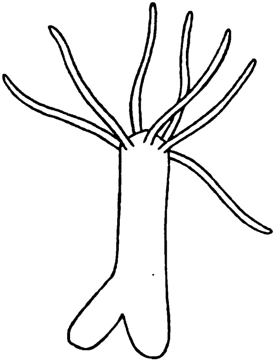

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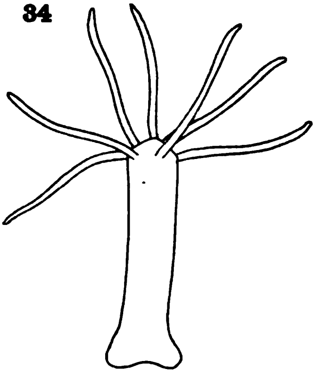

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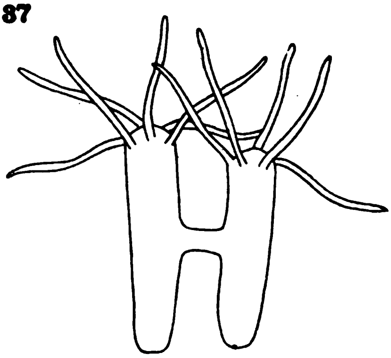

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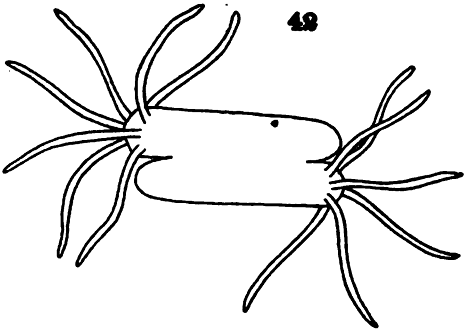

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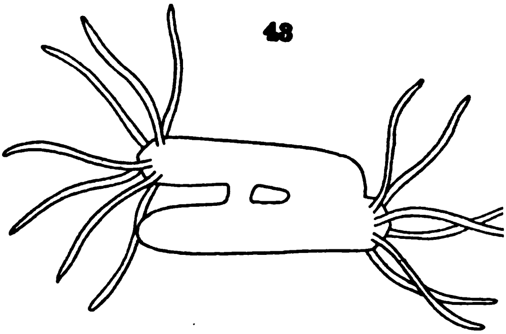

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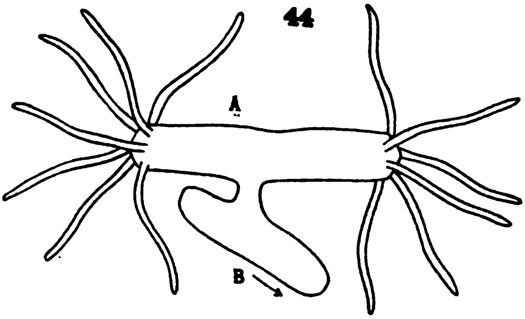

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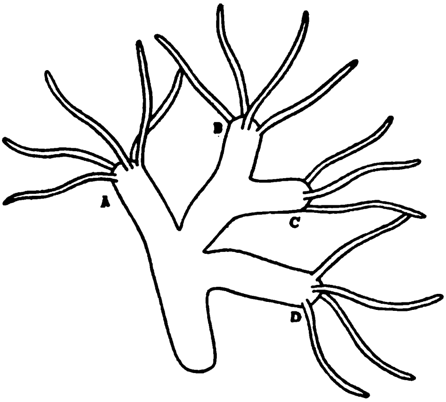

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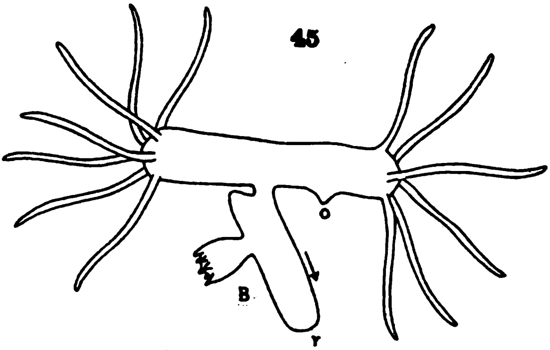

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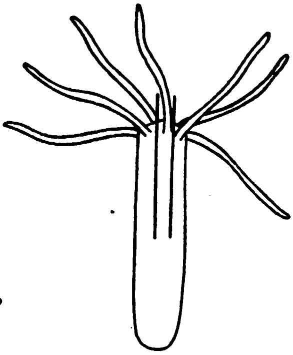

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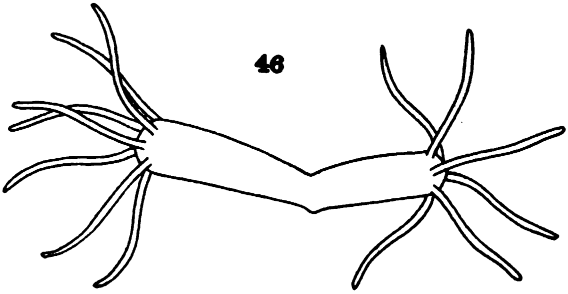

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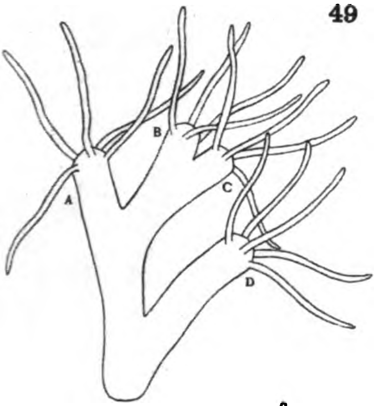

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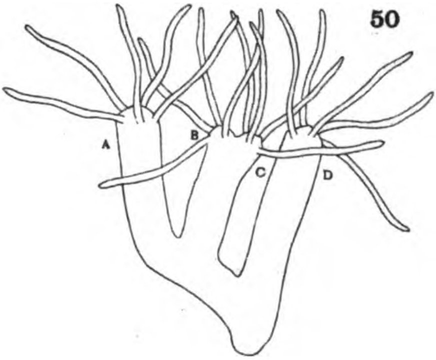

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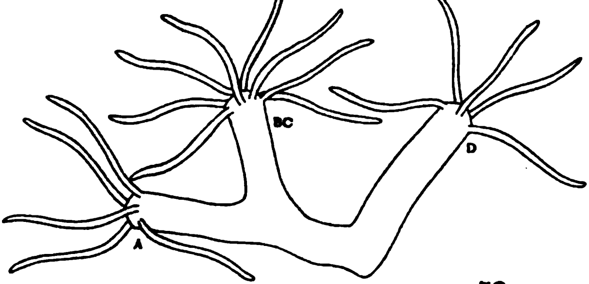

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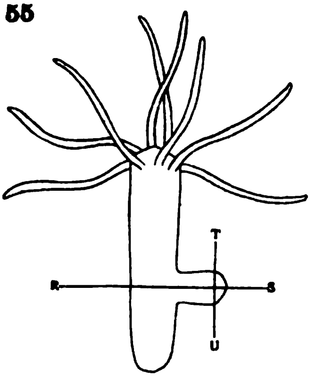

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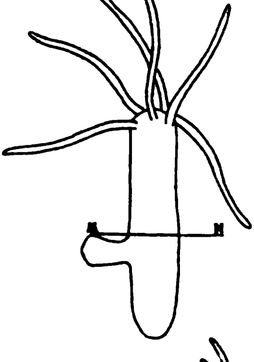

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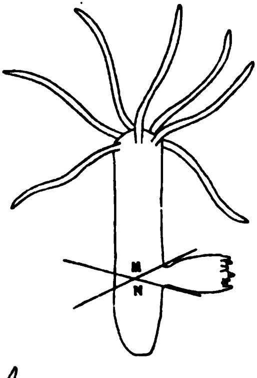

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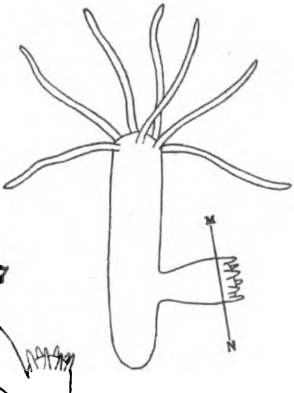

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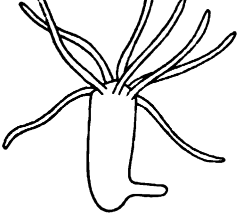

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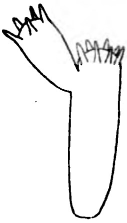

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THE FORMATION OF THE NOTOCHORD IN THE AMPHIBIA.

HELEN DEAN KING.

A study of the mode of development of the notochord in the common toad, *Bufo lentiginosus*, and of the frog, *Rana palustris*, has brought to light certain points that have a bearing on the formation of the same structure in related groups. A vast amount of work has already been done along this line, yet a wide difference of opinion exists among embryologists regarding the origin of the notochord in the Amphibia. It is hoped that the results recorded in the present paper may help to clear up this question.

The material used was fixed in corrosive-acetic (5° glacial acetic acid), and the sections were stained on the slide with a mixture of borax-carmine and Lyon's blue as described in a previous paper (King, 11). This stain gives particularly good results when it is used on freshly preserved material, as then all of the nuclei become dark red, the ectoderm and mesoderm appear dark blue, while the yolk cells take but a pale blue tint and, therefore, are easily distinguished from the other cells. This sharp definition of the tissues was of great assistance, particularly in the study of the sections of *Bufo*. All of the drawings given in the present paper were outlined with the aid of a camera lucida.

BUFO LENTIGINOSUS.

When the circular blastopore is closing in, the mesoderm, already differentiated from the other tissues, forms a continuous sheet of small, angular, slightly pigmented cells across the dorsal wall of the archenteron. In the middle and also in the anterior part of the embryo, the mesoderm is entirely separated from the ectoderm above as well as from the endoderm beneath it. In the region just in front of the blastopore, the mesoderm is also distinct from the ectoderm, but it is united for some distance with the cells forming the dorsal wall of the archenteron. At this stage of development there is first noticed, in the middle of the embryo, a pronounced thickening of the mid-dorsal mesoderm (Fig. 1, *N*), which extends only over a few sections at first and is continuous with the lateral mesoderm on either side. When the blastopore is nearly closed, the thickened portion of the mesoderm is cut off from the lateral mesoderm to form the notochord, the line of separation coming in at about the points marked *XX* in Fig. 1. As the embryo elongates, the forward extension of the notochord always takes place in this same way, *i. e.*, by the cutting off, laterally, of a portion of the mesodermal layer in the mid-dorsal region so that, from the beginning, the notochord is entirely separated from the ectoderm and also from the endoderm. These observations confirm the statement made in a previous paper (King, 12) that "the anterior part of the notochord is certainly mesodermal in origin."

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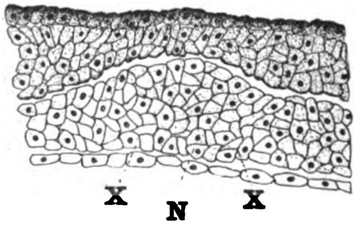

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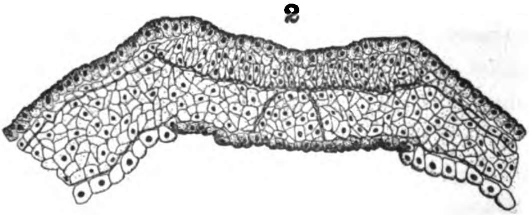
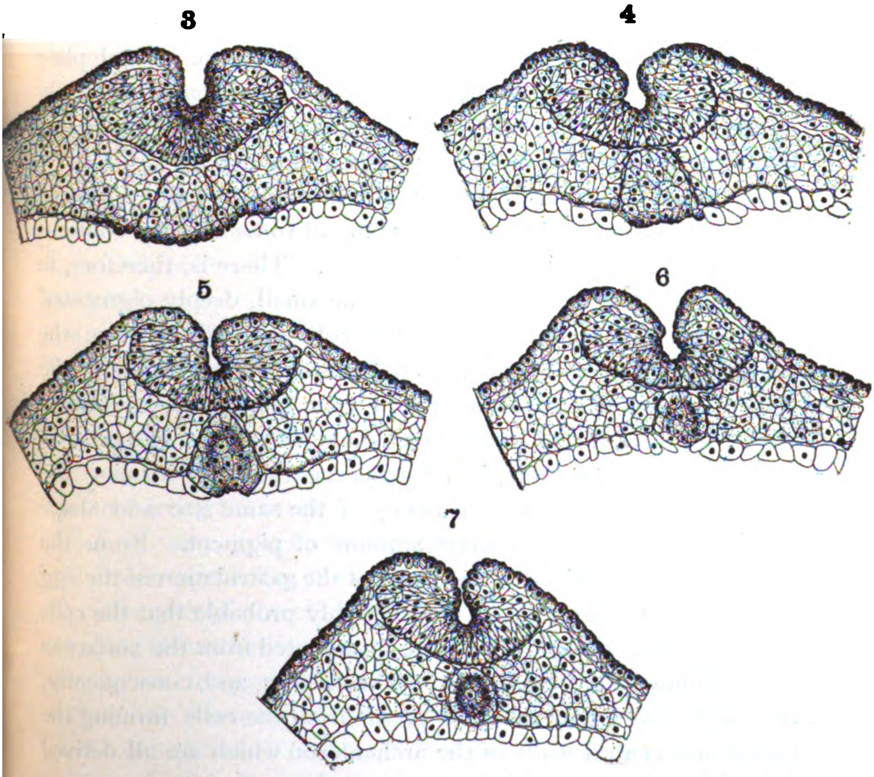


FIG. 1. Part of a medium sagittal section through an egg of *Bufo lentiginosus* in which the blastopore has begun to close. *N*, thickening of mid-dorsal mesoderm which is to be cut off at the points *XX* to form the notochord.

FIG. 2. Part of transverse section through the posterior region of an embryo in which the medullary plate has appeared.

Transverse sections through an embryo in which the medullary plate has just appeared show that, in the anterior region, the notochord is composed of a rounded mass of cells cut off entirely from the surrounding tissues, and appearing much as in Fig. 7. In the posterior region, there is, as yet, no trace of a notochord, and an unbroken layer of cells extends across the dorsal surface of the archenteron, as here the mesoderm is still



FIGS. 3–7. Serial sections from the posterior to the middle region of an embryo of *Bufo lentiginosus* in which the medullary folds are closing.

united with the endoderm as in the earlier stages. In a section made a short distance behind the middle of the embryo (Fig. 2), the notochord appears as a triangular shaped chord of cells, entirely distinct from the mesodermal layer on either side, but closely connected with the cells forming the mid-dorsal wall of the archenteron. In this part of the embryo, as well as more posteriorly, the archenteron is surrounded on its ventral, lateral and lateral-dorsal surfaces by large, rounded, faintly staining yolk cells which contain very little, if any, pigment; the mid-dorsal wall, on the contrary, is formed of a single layer of much smaller, rectangular cells which are very heavily pigmented on the side bordering the archenteron. This layer of cells, which I shall call "the dorsal plate," is broadest in the posterior part of the embryo, where, in transverse sections, it appears as a nearly straight line of cells covering about two-thirds of the mid-dorsal surface of the archenteron. More anteriorly the dorsal plate gradually becomes narrower, until it finally disappears completely in the middle of the embryo. The archenteron in front of this region is entirely surrounded by large yolk cells.

The outer cells of the dorsal plate, instead of grading into the yolk cells as one might expect, are found to be directly continuous with the lower layer of mesoderm. There is, therefore, in this region an abrupt change from the small, deeply pigmented cells of the dorsal plate to the large yolk cells which form the lateral and ventral walls of the archenteron. At no stage in the development of the embryo have I ever found any transitional stages between these two different kinds of cells. The cells of the dorsal plate resemble, in all respects, the cells forming the outer surface of the embryo, being of the same size and shape and containing about the same amount of pigment. From the results which I obtained in my study of the gastrulation of the egg of this species (King, 12), it seems highly probable that the cells composing the dorsal plate were invaginated from the surface of the egg during the formation of the blastopore, and, consequently, they have had a very different origin from the cells forming the lateral and ventral walls of the archenteron which are all derived from the yolk portion of the egg.

When the medullary folds are closing, the mesoderm in the posterior region is still connected, for a short distance, with the cells forming the dorsal wall of the archenteron, and the notochord has not yet extended into this portion of the embryo. Fig. 3 shows a portion of the section through the region where the notochord has just been cut off from the mesoderm. This section corresponds in its position in the embryo with the position of the section of the earlier embryo shown in Fig. 2. The notochord is triangular in shape and is closely connected with the layer of cells forming the mid-dorsal wall of the archenteron. The portion of the dorsal plate directly under the notochord is cut off on either side from the rest of the layer, and to it one can, perhaps, fitly apply the term "chorda-endoderm," since it is destined to become a part of the notochord. At this stage of development, the dorsal plate is much narrower in the posterior region of the embryo than it was before the medullary folds formed (Fig. 2), and it is again found to be directly connected with the lower layer of mesoderm and not with the yolk cells forming the lateral walls of the archenteron.

In Fig. 4, a portion of a section slightly anterior to that shown in Fig. 3, the chorda-endoderm is seen to be the only portion of the dorsal plate bordering the archenteron. The other cells of the dorsal plate have united with the mesoderm, and can only be distinguished from it on account of their position and the fact that they contain somewhat more pigment. The entire dorsal wall of the archenteron, excepting the part formed by the chorda-endoderm, is here composed of large, rounded yolk cells which are evidently growing up from both sides, and thus shutting off all of the cells of the dorsal plate from bordering the archenteric cavity. More anteriorly, as shown in Fig. 5, the yolk cells of the upper wall of the archenteron are still closer together in the middle lines. In this part of the embryo the cells of the chorda-endoderm no longer form a nearly straight line at the lower edge of the notochord, but they have become an integral part of it, and most of their pigment is collected in the form of a pronounced ring around the center of the notochord.

Near the middle of the embryo (Fig. 6), the yolk cells have almost met under the notochord, which is smaller and more rounded than it is in the posterior part of the embryo. A section more anteriorly still (Fig. 7) shows that the yolk cells from the two sides of the archenteron have come together in the middle line under the notochord. As a result, the dorsal wall of the archenteron is composed entirely of a single layer of large yolk cells, and the cylindrical notochord above it is cut off entirely from the surrounding tissues. In the head region, the relation of the tissues is practically the same as that shown in Fig. 7.

When the medullary folds have closed, there is found in the posterior region of the embryo a much narrower dorsal plate than that shown in Fig. 3, as more of the cells have been covered over by the upward growth of the yolk cells from the sides of the archenteric cavity. Anteriorly the dorsal plate grows narrower very rapidly and some distance back of the middle of the embryo the yolk cells have already come to surround the entire archenteron. By the time that the optic bulbs have formed, there is no longer any dorsal plate in the mid-dorsal wall of the archenteron and the notochord has no connection with any of the surrounding tissues.

These results show that the anterior part of the notochord in the embryo of *Bufo lentiginosus* is entirely mesodermal in origin; in the posterior part of the embryo, the greater part of the notochord is also derived from the mesoderm, but there is added to it a single layer of chorda-endoderm from the mid-dorsal wall of the archenteron. Back of the middle region of the embryo, the yolk cells grow up from the lateral walls of the archenteron and unite under the notochord, the cells of the dorsal plate thus cut off from bordering the archenteron, either unite with the notochord or are incorporated into the splanchnic mesoderm.

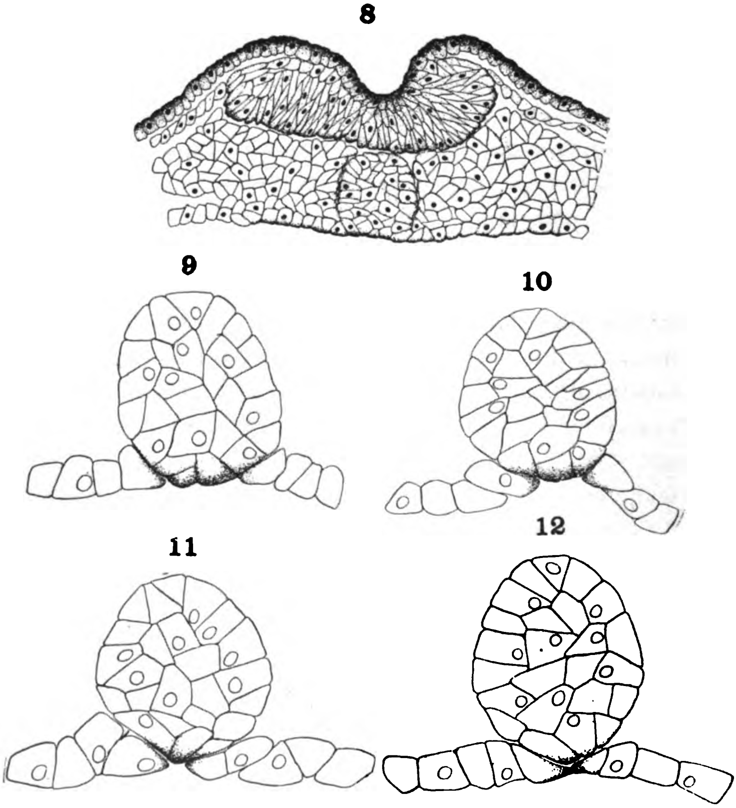
Rana palustris.

In the frog, *Rana palustris*, the notochord is formed at about the same stage of development that it is in *Bufo*, namely, near the end of gastrulation when the blastopore is closing in. As in the embryo of *Bufo*, the notochord first appears in the middle region as a rounded chord of cells cut off from the mid-dorsal mesoderm, and it is separated entirely from the ectoderm and also from the endoderm beneath which forms the dorsal wall of the archenteron. At this stage in the development of the egg, the mesoderm in front of the region where the notochord has been cut off forms a solid layer of cells extending across the dorsal wall of the archenteron and entirely separated from it; the mesoderm back of the notochord also extends in an unbroken sheet across the mid-dorsal region, but in this part of the egg mesoderm and endoderm are connected for a considerable distance on either side of the middle line.

In the posterior part of the embryo the cells forming the dorsal wall of the archenteron do not differ in size, shape, or in power of staining from the mesodermal cells above them, and at the sides of the archenteron they grade into the larger yolk cells forming the ventral and lateral walls. There is a comparatively narrow region in the mid-dorsal wall where the edges of the cells bordering the archenteric cavity are quite heavily pigmented; but the other cells of the dorsal wall contain about the same amount of pigment as do the mesoderm cells above them, and there is no definite dorsal plate of small, deeply pigmented, rectangular cells as in the toad embryo. I can find no evidence that any of the cells of the dorsal wall of the archenteron ever form a permanent union with the mesoderm.

When the medullary folds are beginning to form, the notochord has extended into the posterior region of the embryo and appears as in Fig. 8. It is a more rounded structure than is the notochord of the toad embryo at a corresponding stage of development (Fig. 3), yet it, too, is closely connected with the endodermal layer of cells forming the mid-dorsal wall of the archenteron. As shown in Fig. 8, the lateral mesoderm and the endoderm of the archenteric wall are connected for some distance on either side of the notochord. The cells of both of these tissues have the same general characteristics, and there is no sharp distinction between them as in the embryo of *Bufo*. As all of the cells in the dorsal part of the embryo have the same power of staining, it is not easy to follow the changes that take place, even with an abundance of material for study. Not until I had made camera drawings of a complete series of sections through the posterior region of an unusually favorable embryo was I able to tell with certainty how the notochord is formed. Four of these drawings (from the same embryo as Fig. 8) are reproduced in Figs. 9–12. For the sake of clearness only the dorsal wall of the archenteron and the notochord are shown. In all of the sections the mesoderm is entirely cut off from the notochord, and also from the endoderm beneath it.

A short distance in front of the region shown in Fig. 8, almost all of the pigment in the mid-dorsal wall of the archenteron is found to be massed in the outer edges of a very few cells which are entirely cut off from the other cells of the archenteric wall and are attached to the lower surface of the notochord (Fig. 9). These few cells are undoubtedly comparable to the layer of chorda-endoderm found in the mid-dorsal wall of the archenteron in the toad embryo, and, therefore, the same term may fitly be applied to them. More anteriorly (Fig. 10) there is a noticeable upward bend in the mid-dorsal wall of the archenteron, and it appears as if the notochord with the chorda-endoderm cells is either pulling in or being pushed in from bordering the archenteric cavity, while the cells of the dorsal wall of the archenteron



FIGS. 8-12. Serial sections from the posterior to the middle region of an embryo of *Rana palustris* in which the medullary folds are closing.

on either side of the notochord are coming together under the notochord. A few sections beyond (Fig. 11), the notochord is almost entirely cut off from the archenteron, as only one or two heavily pigmented cells lie between the two parts of the dorsal endoderm. In the middle region of the embryo (Fig. 12), the endodermal cells have united under the notochord and the notochord is a rounded chord of cells entirely separated from the surrounding tissues.

In *Rana palustris*, therefore, as well as in *Bufo lentiginosus*, the notochord is composed entirely of mesoderm in the anterior part of the embryo, and of mesoderm and chorda-endoderm in the posterior region. The early stages in the formation of the notochord are very similar in the two species; but in *Rana* there is no upward growth of yolk cells as in *Bufo* to form the permanent dorsal wall of the archenteron.

Most of the embryologists who have studied the early development of the Urodela agree with Jordan (10) who describes the formation of the notochord in the common newt as follows: "The cells of the median dorsal wall of the archenteron assume a somewhat columnar form and are gradually pushed up and pinched off until they are completely separated from the endoderm and come to lie above it in the mid-line." This view is held by Hertwig (7), Scott and Osborn (20), Field (5), Eycleshymer (4), Brachet (2), and Schwink (19).

Lwoff (13) is, perhaps, the most prominent of those who oppose this view. In his study of *Axolotl*, Lwoff finds that the mesoderm and the notochord are derived from cells invaginated from the surface of the egg at the blastopore rim, and he states: "Bei den Urodelen bildet sich die dorsale Wand des Darmes, ebenso wie bei Petromyzon, verhältnissmässig spät, nämlich nachdem die Chorda sich von den seitlichen Mesodermplatten gesondert hat. Die Entodermzellen wachsen von rechts und links einander entgegen, vereinigen sich unter der Chorda und bilden aufsolche Weise die dorsale Wand des Darmes." This description of the manner in which the permanent dorsal wall of the archenteron is formed in the *Axolotl* agrees remarkably well with the results of my investigations on *Bufo*. Lwoff's summary of the results of his study of the Anura based on an investigation of the early development of *Rana*, is in part as follows: "Bei den Anuren liegen insofern anderen Verhältnisse vor, also hier die Zellen, welche die dorsale Wand des Darmes bilden, von Anfang an vorhanden sind ale eine Zellenreihe und zwar als eine untere Zellenreihe jener Anlage, aus welcher die Chorda entsteht." Lwoff and I are therefore in accord in believing that in *Rana* there is no upward growth of the yolk cells from the lateral walls of the archenteron to form the mid-dorsal wall.

There is great diversity of opinion concerning the manner of the formation of the notochord in the Anura; and, considering the careful work that has been done in this line, it seems highly probable that the process is not as uniform in this group as it is in the Urodela.

Goette (6), from his study of the development of *Bombinator igneus* concludes that in this species a central chord of mesoblast in the mid-dorsal region of the embryo separates from the two lateral sheets to form the notochord. This view is supported by the later investigations of Schultze (18), and Morgan (15) who worked on different species of *Rana*.

In a paper on the development of the middle germ layer in *Rana temporaria*, Hertwig (8) gives a number of figures of the posterior part of the embryo that bear a striking resemblance to those I have drawn of a similar region in the embryo of *Bufo lentiginosus*. Hertwig believes, however, that the entire notochord in the Anura as well as in the Urodela, is derived from a chorda-entoblast which at the sides of the archenteron pass into the endoderm cells forming the lateral walls. Field (5), from his investigations on *Rana temporaria* and on *Bufo vulgaris*, agrees with Hertwig regarding the manner of formation of the notôchord, as do Robinson and Assheton (17) who worked on *Rana temporaria*. Balfour (1) also inclines to the same opinion, although he states that his evidence for so doing is not entirely conclusive.

As a result of his study of the early development of *Bombinator igneus*, Perenyi (16) advances still another theory regarding the formation of the notochord. He states that, when the blastopore closes in, "die vertikal nach innen vordringenden Zellen der Deckzellen, welche zwischen beiden Teilen des Mesoderms liegen einander berühren und sich auf der dorsalen Seite von den äussersten Zellen abzuschnüren beginnen." In this way a rod of cells is cut off from the inner layer of ectoderm to become the notochord. I know of no other investigator whose results agree with those of Perenyi.

The results which Schwink (19) has obtained from his investigations on *Rana temporaria* and *Bufo vulgaris* are very similar indeed to those which I have recorded in the present paper for *Rana palustris* and *Bufo lentiginosus*. According to Schwink, the anterior portion of the notochord in *Rana temporaria* is entirely mesodermal in origin, while the posterior part has added to it a single layer of chorda-endoderm from the dorsal wall of the archenteron, the endoderm cells at the side of the notochord growing under and uniting in the mid-dorsal line. In *Bufo vulgaris* Schwink finds that the dorsal wall of the archenteron is composed of deeply pigmented cells which, at the sides of the archenteron, pass into the larger yolk cells, although he states that in some cases it appears "dass die hier liegenden Entoblastzellen aus dem bisherigen Verband scheiden um in den Mesoblast aufgenommen zu werden." Concerning the formation of the dorsal wall of the archenteron in the posterior part of the embryo Schwink states that, "hier von beiden Seiten Darmentoblastzellen gegen die Mittellinie streben und dass dadurch Zellen, die vorher den Darm dorsal auskleideten, mit zur Bildung der Chorda verbraucht werden." This agrees exactly with what I have found to occur in the posterior region of the embryo of *Bufo lentiginosus*.

Brauer's (3) studies on the development of the Gymnophiona show that, in the posterior region of the embryo, the upper wall of the archenteron is at first formed of cells which have been invaginated from the surface. These "animal cells" are sharply marked off from the yolk or "vegetative cells" which form the side walls of the archenteron. In the anterior part of the embryo, the archenteron is extended by its connection with the segmentation cavity which is bounded entirely by yolk cells. At an early stage of development, therefore, the dorsal wall of the archenteron in the anterior region of the embryo is composed of vegetative cells, while in the posterior region it is formed of cells invaginated from the surface as I have found to be the case in the embryo of *Bufo lentiginosus*. At a later stage of development, vegetative cells grow up from the sides of the archenteron, and gradually cover up the invaginated animal cells which now form an unbroken sheet of mesoderm across the dorsal wall of the archenteron. A portion of this mesoderm in the mid-dorsal line is subsequently cut off from the lateral mesoderm to form the notochord.

In the posterior region of the embryo of *Bufo lentiginosus* a portion of the dorsal plate of cells which forms the mid-dorsal wall of the archenteron becomes cut off from the rest of the layer to be added to the notochord. If we attempt to trace the origin of this dorsal plate, we find that it is composed of cells invaginated from the surface of the egg before there was any division of the cells into ectoderm, mesoderm and endoderm. These invaginated cells form a part of the upper wall of the archenteron for a comparatively short period of development only, and those of the cells that are subsequently added to the splanchnic mesoderm soon lose their identity entirely, and cannot be distinguished in any way from the other cells of the mesoderm. The later history of the chorda-endoderm cells I have not followed.

As the endoderm cells that grow up from the sides of the archenteron and meet under the notochord are unquestionably derived from the yolk portion of the egg, the archenteron eventually becomes lined throughout its whole extent with yolk cells, and, therefore, the result is the same as if the archenteron was originally formed by a splitting between yolk cells as is believed to be the case by Robinson and Assheton (17), Houssay (9) and Moquin-Tandon (14).

According to Morgan, Wilson (21), Eycleshymer and others, there is an invagination of surface cells at the dorsal lip of the blastopore during the gastrulation of the frog's egg, and these invaginated cells come to form a part, if not all, of the dorsal wall of the archenteron in the posterior region of the embryo. In subsequent development, as the studies of Schwink and of myself show, these invaginated cells are not covered over by an upward growth of yolk cells from the lateral walls of the archenteron as is the case in the toad embryo. A few of these cells are added to the notochord, the rest, as far as I have been able to determine, remain as part of the permanent dorsal wall of the archenteron. I have never seen a section of an embryo that would warrant my stating that some of these cells become added to the mesoderm, although in the posterior region of the embryo endoderm and mesoderm are connected for a much longer time than they are in the embryo of *Bufo*.

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Notes on Regeneration in Stentor coeruleus.

By

N. M. Stevens.

With 55 figures in text.

Eingegangen am 17. März 1903.

The problem suggested to me by Prof. T. H. MORGAN, — to determine more definitely than had been done the mechanism by which a new peristomal field is formed in Stentor, — proved to be difficult of solution, but some light was thrown upon this question by comparison of fission with regeneration. Incidentally other results were obtained which seem to merit publication.

Method. — The stentors were cut with a sharp scalpel in a paraffined watch-glass, and the pieces isolated in watch-glasses filled with either the culture fluid or with fresh spring water. At times development was delayed by placing the cultures near an open window during the night.

Formation of a new peristomal field. — In studying the development of a new peristome and frontal field, several points were considered, — movements of the peristomal band, shifting of the cytoplasm, and origin of the narrower stripes of the frontal field.

When a stentor is cut transversely into two parts, as in Fig. 1 *a—b*, the new peristomal band in the proximal piece appears in the position shown in Fig. 2, lying somewhat obliquely across the stripes, ventral to the ramifying region[1]), as in fission, but with its aboral end at or near the center of the distal end of the piece, where the stripes are gathered together by the closing of the wound (Fig. 3). A little later the aboral end of the band begins to advance around the dorsal side of the central terminal point (Fig. 4); the band lengthens by increasing the distance between the membranellae, and the gathered ends of the stripes on the dorsal side straighten out along the dorsal margin of the band. The ventral stripes, meanwhile, are carried along nearly parallel with the band, and are slightly stretched and narrowed (Figs. 4 and 5). At this time there is no multiplication of stripes within the prospective frontal field, and the number of stripes included varies with the size of the piece.

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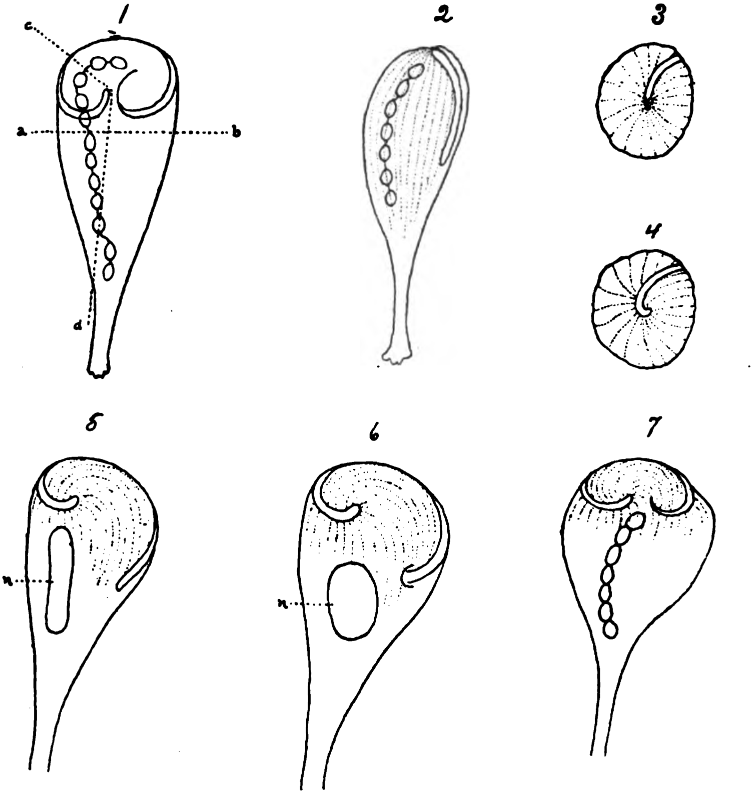
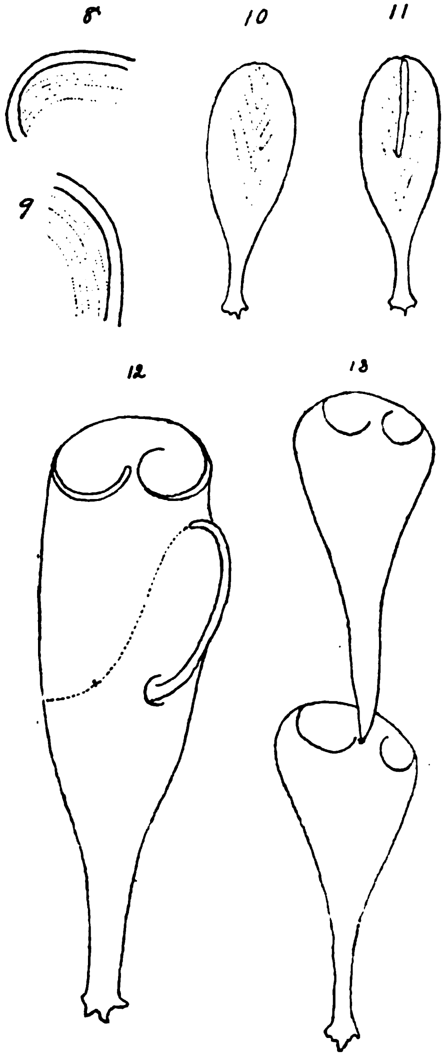
[1]) The »Verästelungszone« of SCHUBERG ('90), a region on the left side of Stentor coeruleus where the ventral stripes run off obliquely from a single dorsal stripe. This departure from the usual parallel relation of the stripes is the result of the oblique constriction which separates the two daughter stentors in fission (JOHNSON, '93).

Figs. 1—7.



The aboral end, having formed its portion of the new frontal field, remains stationary or nearly so, while the invagination which forms the pharynx takes place at the oral end of the band (Fig. 6). In the course of the latter process, the stripes of the frontal field are drawn into the gullet to form a part of its lining, and are thus stretched and narrowed still more than at the aboral end. A rolling of the ventro-lateral surface below the band into the gullet brings the mouth-opening nearer and nearer to the aboral end of band. Simultaneously with the invagination of the pharynx, a shifting of the cytoplasm occurs, changing the form of the anterior portion of the stentor so as gradually to throw the frontal field, now nearly enclosed by the peristomal band, into a terminal position (Fig. 7). The mouth is later brought somewhat nearer to the aboral end of the band, apparently by the deepening of the pharynx.

Figs. 8--13.



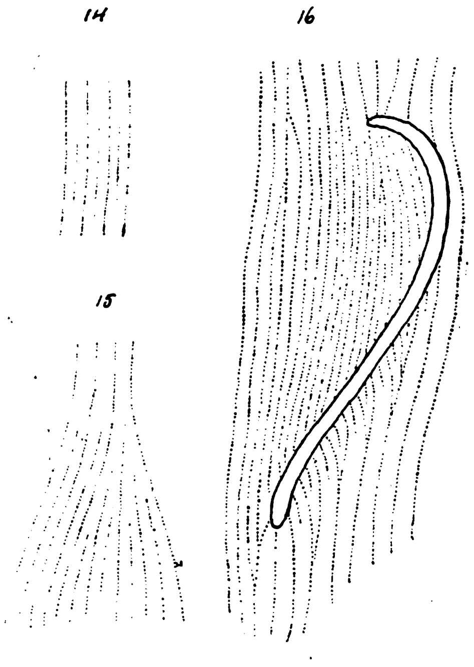
The number of pigmented stripes in the frontal field is increased later by intercalation of new light stripes, dividing the blue stripes. Johnson ('93) and Balbiani ('91 a) suggested the probability that this was the explanation of the narrower stripes in the frontal field, but neither observer had seen the process taking place. Such division of the pigmented stripes as appears in Figs. 8 and 9 may often be observed the next day after regeneration of the peristome.

The nuclear changes connected with regeneration have been fully described by Balbiani ('91) and Johnson ('93).

If the posterior part of a stentor, cut as in Fig. 1 *a—b*, is then cut longitudinally in a dorso-ventral plane, the piece which does not contain the ramifying region will form a new peristomal band along the distal portion of the longitudinal seam (Figs. 10 and 11).

Pieces from the dorsal side of the animal have a very few broad stripes included in the new frontal field at first, and therefore afford a better opportunity for observing multiplication of the stripes, than pieces in which the field is formed from the stripes of the ventral side, which are always narrower. In one case, only eight stripes were included in the field. The next morning the two outer blue stripes were being divided by the insertion of new light stripes, and division of other stripes was observed later in the day. The following morning there were twelve blue stripes, and more light stripes were coming in.

Figs. 14—16.



In fission, which is quite fully described by Johnson ('93, Pl. XXIV Figs. 26—37), the principal factors which bring the lateral peristomal band into a terminal position are 1) the invagination of the pharynx simultaneously with considerable protoplasmic shifting to the region of the new peristome, and 2) what seems to me of prime importance, the rapid contraction of the oblique constriction line (Fig. 12), which draws the aboral end of the band down to a plane nearly parallel to the mouth-opening (x in Fig. 12), and cuts off the distal stentor (Fig. 13), allowing the aboral end of the band to extend across the dorsal stripes around to a point near the gullet, much as it does in regeneration.

In fission, I find that multiplication of the pigmented stripes by introduction of new light stripes may begin several hours before the peristomal band appears (Fig. 14). Later stages are shown in Figs. 15 and 16. In Fig. 16, there are indications that division of the colored stripes had gone quite far before the band appeared, and that the opening through the ectosarc cut across some of the newly formed light stripes. Fig. 15 shows not only insertion of many new light stripes but increase in the circumference of the body of the stentor in the region of the new peristome. These stages (Figs. 15 and 16) were observed before the membranellae had formed from the rift of exposed endosarc. Further division of stripes was observed in a proximal stentor after fission was completed, and it is probable that there is considerable variation in different individuals as to the time when the new stripes come in.

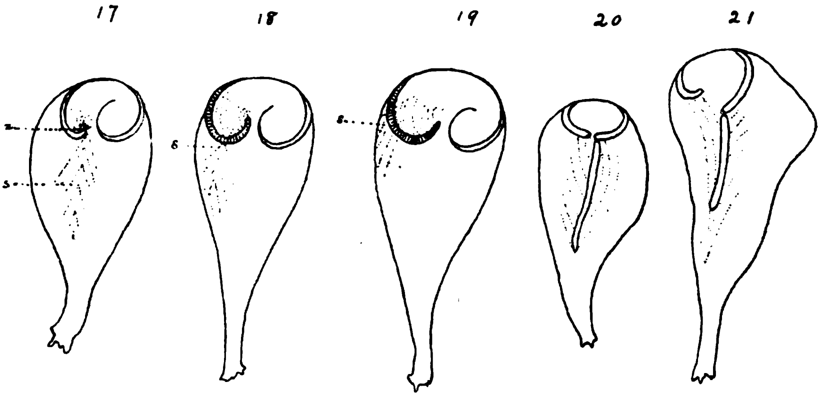
Repair and regeneration in injured peristomes. — In cases where a stentor was cut longitudinally through the peristomal field, as in Fig. 1 *c—d*, without injuring the pharynx, the part containing the pharynx closed so as to bring the cut end of the band around near the oral aperture, and no regeneration either of the whole peristome or of the pharynx occurred. The part of the old band left in the piece did, however, increase considerably in length in the course of 72 hours, whether merely by separation of the membranellae, or by intercalation, or by addition of new ones, I was unable to determine with certainty. In several cases the seam (*s*) coincided exactly with the cut end of the band, an hour or so after cutting (Fig. 17). Later the end of the band gradually extended 12, 20, 25 or more membranellae beyond the seam; but whether this was due to formation of new membranellae at the end of the band or to a forward movement and lengthening of the band, as in the development of a new peristome, is not certain, since it was impossible to count all of the membranellae in the band. The membranellae at the tip of the band, as it advanced beyond the seam, appeared to be smaller than those behind, but no rift in the perisarc, such as is seen in ordinary regeneration or fission, could be detected. The extension or stretching of the band was so great that in many cases after two or three days such stentors could be distinguished from normal ones only by the irregularity of the stripes where the cut had closed, and my impression is that new membranellae must have been added to those present when the animals were cut in two. These specimens were kept in the cold at night to prevent physiological regeneration from occurring unobserved.

The individual shown in Figs. 17—19 was cut at 8.45 A. M. Jan. 12th. Sketch 17 was made at 10.00 A. M. At that time the seam on the ventral surface coincided with the cut end of the band,

and the cut end of the stripes of the frontal field were gathered up at the same point (x). At 4.45 P. M., there were 5 membranellae beyond the seam; Jan. 13th at 9.00 A. M., 12; and Jan. 14th at 8.30 A. M., 20, and at 4.00 P. M., 25. The next morning the seam was still further around towards the dorsal side, but it was impossible to count the membranellae.

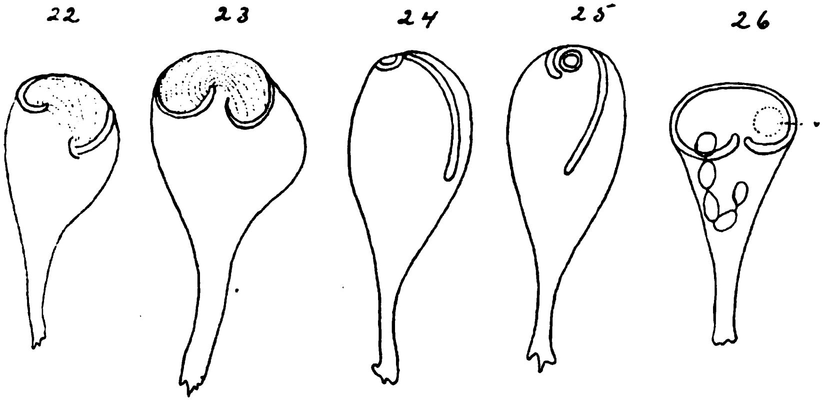
The other half of the stentor — including the aboral half of the peristomal field — usually closes so as to bring the two ends of the band nearly together (Fig. 20). A new band appears along the seam from 2—10 hours after cutting. The aboral end of the new band is always in contact with the cut end of the old band. The length of the new band varies with the length of the part of the old band left in the piece; in pieces having all of the old band aboral to the pharynx region, the new band is noticeably shorter than in pieces of the same size in which only a small portion of the old band is present. The length of band regenerated seems to be proportional to the length of band removed, or to the length required to complete a peristome of normal size for the piece (Figs. 20 and 21) (Morgan, '01).

Figs. 17—21.



Regeneration in these pieces is very evidently equivalent to physiological regeneration as described by Johnson. The part of the old peristomal field present opens out (Figs. 22 and 23), the aboral end apparently retreating across the stripes included in the new frontal field. A peristome, normal in form, but with a divided frontal field, is the final result. There is no formation of a new ramifying zone, but the normal process of physiological regeneration comes into play; and instead of a new rift in the ectosarc being formed, the new band forms at the line of union of the two cut edges, and the whole of the part of the old band present is made use of in forming a new peristome. In all probability the ectosarc of the cut edges in the region where the band develops never heals perfectly. In many cases a white stripe was observed in this region several hours before the membranellae could be detected in a side view.

In several cases, — three out of one series, — where large stentors were cut longitudinally, the oral part of the peristome enlarged and remained functional as described; but within from two to four hours after cutting, a fission-band appeared and developed more slowly than normally. In one specimen, cut at 8.30 A. M., a new band was seen at 12.30, the nucleus was beginning to concentrate and the pharynx to invaginate at 6.00 P. M. At 9.15 P. M. fission was practically complete, but at 11.00 the two individuals had not separated. The natural interpretation of these phenomena is that at the time of cutting, the processes which lead to fission had already been set up in the cell, and that, although a third or a half of the cell, including a part of the peristome and a part of the nuclear chain, was removed, the process of fission went on. The concentration of the nucleus and the invagination of the pharynx were, however, considerably delayed by the operation. The aboral portion of the band lengthened meanwhile, the membranellae becoming more widely separated than is usual before constriction of the body begins. Johnson mentions one similar case where he cut



Figs. 22–26.

off a small piece of the distal end of a stentor and subsequent fission gave a larger distal and a smaller proximal individual.

In a few cases where a part of the border of the pharynx was removed, the cut ends came together and no physiological regeneration followed; but any injury to the oral half of the peristome usually resulted in regeneration on the same or the following day.

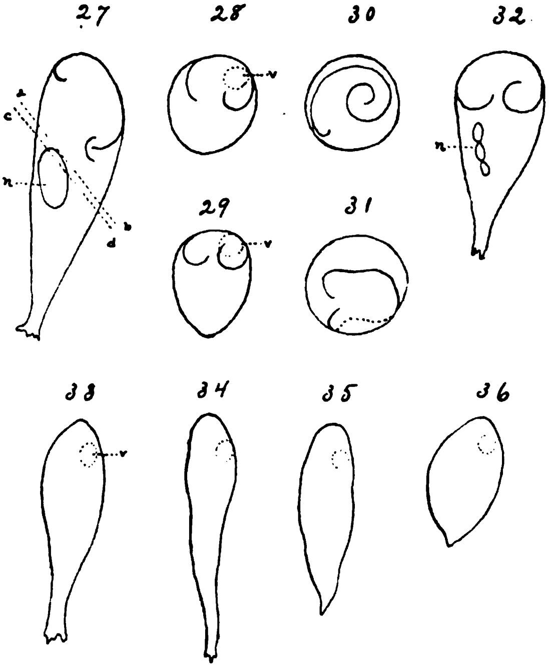
In pieces from which the greater part of the peristome was removed, leaving only a part of the oral region, a new band appeared touching the old one as in physiological regeneration (Fig. 24), but the aboral end moved around the remains of the old gullet (Fig. 25), which eventually sank into the endosarc and disappeared. In only one case was the aboral end of the new band observed to coil ventral to the old gullet.

Occasionally pieces from the aboral side of a stentor do not regenerate at once, but the irregular piece remodels itself into the form of a normal animal with a new contractile vacuole but no pharynx. Fig. 26 shows such a piece 30 hours after cutting.

Whatever may be the relation between physiological regeneration and fission (JOHNSON regards them as closely related and even mutually convertible processes), regeneration in cases of artificial section, or merotomy, seems to be an adaptation of the process of physiological regeneration to the conditions existing in the piece. If a part of the old peristome that can be made use of in forming a new one remains, it is retained (Figs. 20—23); if not, it is absorbed. In case the old peristome is wholly removed, the new band forms a wholly new peristome as in fission, but the stripes included are at first undivided as in physiological regeneration, while in fission division of the stripes and enlargement of the prospective peristomal field is the first indication of the formation of a new individual.

Non-nucleated pieces. — In connection with this work, I was able to confirm by a different experiment GRUBER'S ('85) statement, that a peristome once begun will be completed after the nucleus is removed. The peristome of the stentor was removed by a transverse section (Fig. 1 *a—b*), and the posterior part allowed to regenerate to a stage in which the nucleus was in a concentrated condition. An oblique section was then made as in Fig. 27 *a—b*. The closing of the cut surface of course threw the peristomal field into an unnatural position, but in every case the invagination of the pharynx went on, and the oral and aboral ends of the peristome came into normal relations to one another (Figs. 28 and 29). In some cases the piece elongated slightly (Fig. 29), but never formed a foot by which to attach itself, and usually remained nearly spherical. In one specimen the pharynx gradually disappeared and 24 hours after the piece had been cut off, the band was coiled as in Fig. 30. In others the field became very irregular as in Fig. 31, — due to lengthening of the band over a limited surface, the piece being no longer capable of assuming the characteristic stentor form, or of increasing in size. The contrast in behavior between such non-nucleated pieces and others containing a very small piece of the nucleus is seen by comparing Figs. 28 — 31 with Fig. 32, the latter piece having been cut as in Fig. 27 *c—d*, and containing a small piece of the nucleus.

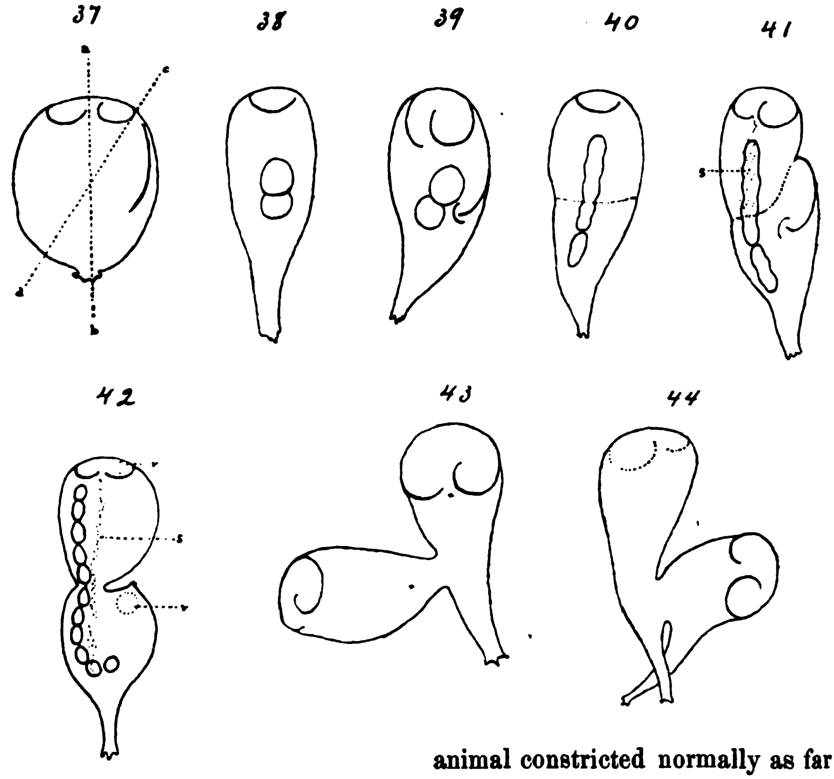
Figs. 27—36.

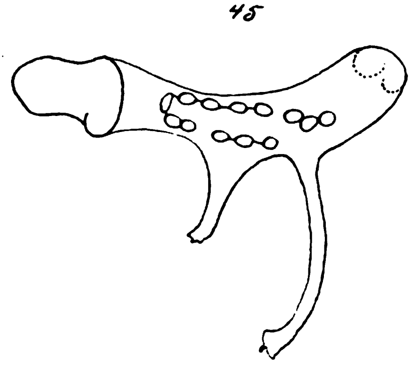


Posterior non-nucleated pieces attached themselves by the foot already present, formed a functional contractile vacuole, contracted, expanded and swam about like normal stentors, but never developed a new peristome. After about two days they lost power of attachment, and gradually assumed a permanently contracted condition (Figs. 33—36).

Longitudinal section in early stages of fission. — While carrying on these experiments I accidentally sectioned a stentor in which a fission band had appeared (Fig. 37 *a—b*). Fig. 38 shows the aboral half, and Fig. 39 the oral half, three hours after cutting. Half an hour later I was much surprised to find that the usual constriction line had become visible not only on the half possessing the new peristomal band (Fig. 41), but on the other half also (Fig. 40). In both pieces the constriction line was in normal position for the whole stentor before cutting, but stopped about two stripes short of the seam where the cut edges had united. The oral half of the animal constricted normally as far as the constriction line extended, but the individuals remained connected in the unconstricted region near the seam. Five and seven hours later respectively, they appeared as in Figs. 43 and 44 (x—x in Fig. 43 indicating the points where the individuals should have separated). The aboral half constricted simultaneously with the oral half (Figs. 40 and 42), but the two individuals partly fused together again and died without developing further. Nuclear changes occurred simultaneously in the two halves as shown in Figs. 38 and 39, 40 and 41.

Figs. 37—45.

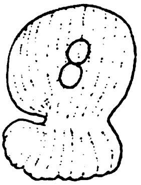




A considerable number of individuals were similarly sectioned in about the same stage, and it was found that without exception, and in spite of great irregularities in the closure of the edges of the cut surface, concentration of the nucleus and constriction of the two halves in a predetermined plane occurred simultaneously. In cases where the longitudinal cut edges united regularly, the constriction in each piece usually resulted in the production of two separate stentors, regeneration occurring in the two aboral individuals. More often, however, the pieces were so folded in closing that separation of the individuals was impossible and double monsters resulted. In some cases the partly separated stentors fused together again; in others such double stentors as Fig. 45 separated 24 hours or more after section, and formed two normal animals, Figs. 46 and 47 show constriction in irregularly folded aboral pieces. The part *a*, in the individual shown in Fig. 47, constricted off as a non-nucleated piece which attached itself by the foot (*f*) and lived for some time. In one case of incomplete division (Fig. 48), cut as in Fig. 37 *c—d*, the aboral half developed an irregular peristome in the proximal portion (Fig. 50 *p*). This peristome was at first in a nearly reversed position, but shifted to the position indicated in Fig. 51. In another similar piece the proximal individual gradually shifted a large part of its

Figs. 46 - 51.

46



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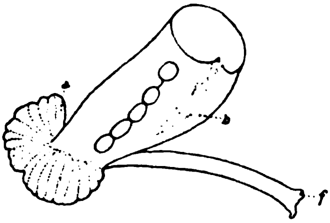

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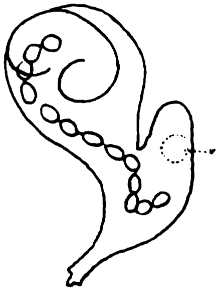

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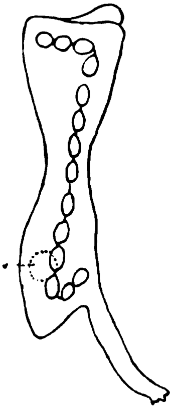

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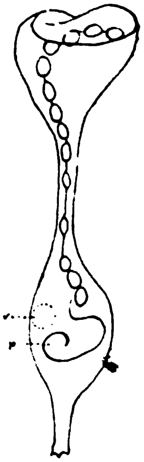

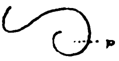

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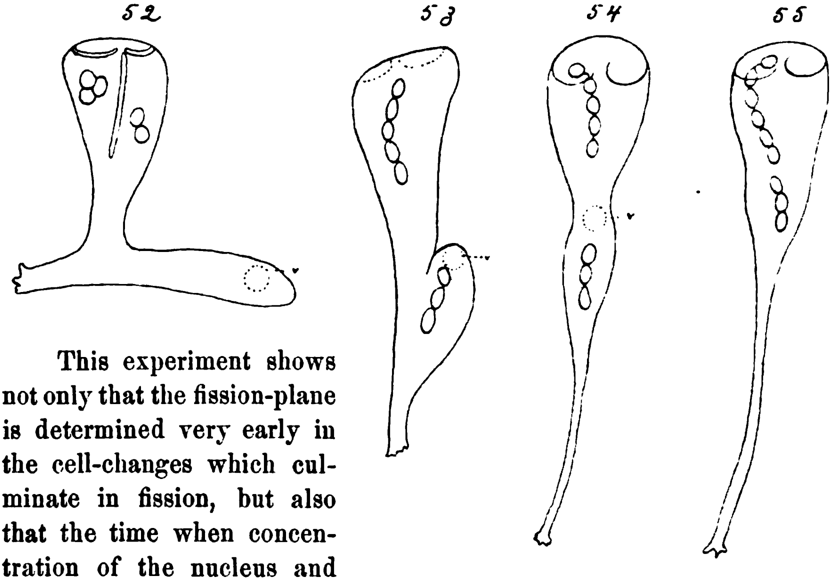
cytoplasm and its nucleus into the distal one, so as to form a single normal stentor (Figs. 52—55). In one case the whole of the already concentrated nucleus was included in the aboral half, and constriction went on as usual in the oral half, the new peristome assuming as nearly normal position as was possible in the folded condition of the piece. The fate of this piece was not followed further, but it was killed and stained three and one half hours after cutting, to be sure that no part of the nucleus was present.

Figs. 52—55.



This experiment shows not only that the fission-plane is determined very early in the cell-changes which culminate in fission, but also that the time when concentration of the nucleus and constriction of the body shall occur, is also determined at an early stage, and is not changed by artificial division of the cell. Although these two phenomena are always correllated in time with the invagination of the pharynx, they are not dependent for their occurrence on a particular stage in the development of the peristome, since they occur simultaneously in the two halves of the stentor, three hours after separation. In other words, the three correllated phenomena, — 1) formation of a new peristome, 2) concentration and renodulation of the nucleus, and 3) fission of the body of the stentor, must be, not separate phenomena following one another in sequence, but parts of one process in which the whole cell is concerned, and which is, as it were, planned and determined before there is any external evidence that it is to occur.

Something similar was shown in my experiments on eggs of Echinus microtuberculatus (Stevens, '02), where pieces cut soon after the daughter-plates of the first segmentation spindle had separated, and including no part of the spindle — no chromatin and no asters — nevertheless showed one division in the plane of the first cleavage of the remainder of the cell. Whether this occurred at the same time as the division of the part of the egg containing the spindle was not observed ('02, Pl. XIII Fig. 12).

Summary.

1) In the formation of a new peristomal field during fission of Stentor coeruleus, intercalation of new light stripes begins, before the peristomal band appears and may or may not be completed before the individuals separate.

2) In physiological regeneration and in regeneration after merotomy, the number of stripes in the frontal field is increased by intercalation of new light stripes, beginning several hours after the field has assumed its normal position.

3) Invagination of the pharynx, oblique constriction of the body, movement of the aboral end of the peristomal band across the dorsal stripes cut off by fission, and shifting of the cytoplasm are the chief factors concerned in bringing the lateral peristomal field of a dividing stentor into a terminal position. In merotomy there is, of course, no constriction, but the aboral end of the band advances around the distal end of the piece.

4) Regeneration after merotomy appears to be a modification or an adaptation of the process of physiological regeneration.

5) In pieces, from the right and from the dorsal sides of a stentor, containing no ramifying region, the new band develops along the distal portion of the line of union of the cut-edges. If an aboral portion of the old peristome is present, it forms a part of the regenerated peristome.

6) There is some evidence that new membranellae are formed at the cut end of a peristome from which the aboral end has been removed.

7) Non-nucleated parts of either regenerating or dividing stentors complete the formation of a peristome as stated by Gruber.

8) If a stentor whose fission band has just appeared be divided by a dorso-ventral longitudinal section, concentration of the nucleus

and constriction of the body will occur three or four hours later, simultaneously in the two halves.

Bryn Mawr College, Feb. 27, 1903.

Zusammenfassung.

1) Bei der Entstehung eines neuen Peristomfeldes während der Theilung von Stentor coeruleus beginnt die Intercalation neuer heller Streifen vor der Erscheinung des peristomalen Bandes und kann vor der Trennung der Individuen vollendet sein oder nicht.

2) Bei der physiologischen Regeneration und derjenigen nach Merotomie wird die Zahl der hellen Streifen im frontalen Felde vermehrt durch die Anlage neuer solcher, welche mehrere Stunden nach der Einnahme der Normalstellung seitens des betreffenden Feldes beginnt.

3) Einstülpung des Pharynx, schräge Zusammenschnürung des Körpers, Wanderung des aboralen Endes des Peristombandes quer durch die dorsalen Streifen, die durch die Spaltung zertrennt wurden, sowie Verlagerung des Cytoplasmas sind die hauptsächlichen Faktoren, welche zur Einnahme der schließlichen Lage seitens des lateralen Peristomfeldes bei einem sich theilenden Stentor in Betracht kommen. Bei der Merotomie findet natürlich keine Einschnürung statt, aber das aborale Ende des Bandes rückt rund um das distale Ende des Stückes vor.

4) Die Regeneration nach Merotomie scheint eine Modifikation oder eine Anpassung des physiologischen Regenerationsprocesses zu sein.

5) Bei Stücken von der rechten, sowie von der dorsalen Seite eines Stentor, welche keine »Verästelungszone« enthalten, entwickelt sich das neue Band entlang dem distalen Theil der Vereinigungslinie der Schnittränder. Ist ein aborales Stück des alten Peristoms vorhanden, so bildet es einen Theil des sich regenerirenden Peristoms.

6) Es ist bis zu einem gewissen Grade sicher, dass sich neue Membranellae am Schnittende eines Peristoms bilden, von dem das aborale Ende entfernt wurde.

7) Kernlose Theile von Stentoren in Regeneration oder in Theilung vervollständigen die Bildung eines Peristoms, wie Gruber nachgewiesen hat.

8) Wird ein Stentor mit eben aufgetretenem Theilungsstreif durch eine dorsoventrale Schnittebene zertheilt, so treten, gleichzeitig in beiden Hälften, drei oder vier Stunden später Verdichtung des Kerns und Zusammenschnürung des Zellleibes ein.

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Aus dem Zoologischen Institut der Universität Würzburg.

On the Ovogenesis and Spermatogenesis of Sagitta bipunctata.

By

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With 2 Plates.

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This study was suggested to me by Prof. **Th. Boveri**, to whom I am deeply indebted for permission to work in his laboratory, during the summer semester of 1902, and for helpful suggestions and criticism.

An investigation of the ovogenesis of *Sagitta* was undertaken: 1) in order to determine what light it might throw on the Reduction Question; 2) to ascertain the relation between the large chromosomes of the germ nucleus and the very small ones in the maturation spindles, as seen by **Boveri** (1890). No satisfactory results were obtained on the first point, but to the second question a definite answer can be given.

As the preparations included sections of the testis, a study of the spermatogenesis of *Sagitta* was also made. The material is particularly favorable, as all stages in the development of both ova and spermatozoa may be found in the same individual.

Method. The animals were preserved whole, and cross-sections cut through the region containing the ovaries and testes. As fixing agents, **Hermann**'s platinum chloride-osmic-acetic mixture, **Flemming**'s strong chrom-osmic-acetic solution, **Boveri**'s picro-acetic, 70 % alcohol, and sublimate acetic were used; but only the latter — concentrated sublimate solution with 2—5 % glacial acetic — gave satisfactory results.

A part of the material was stained in bulk in borax carmine and in **Delafield**'s haematoxylin; but by far the best results were obtained from sections 5—7 μ thick, stained on the slide by **Heiden-**

HAIN's iron-haematoxylin method, and all the figures were made from such preparations.

Ovogenesis.

No careful study of the ovogenesis of *Sagitta* has, so far as I know, been published. O. HERTWIG (1880) and GRASSI (1883) give figures and general descriptions of the ovary, and discuss the function of the so-called sperm-oviduct, the probable point of egress of the eggs from the ovary into the oviduct, and the possibilities in regard to the time and place of the entrance of the spermatozoa; but neither of these authors gives any account of the nuclear changes that occur during the development of the ova. BOVERI (1890) determined the number of chromosomes in the nearly ripe oocytes, described and figured the two maturation spindles, the pronuclei, and the first division spindle.

My material includes embryos 16—18 hours from the time of laying, and adult animals with ovaries in various stages.

The embryos show the two large germ cells preparing for the division which separates the male from the female reproductive cells. Here 18 chromosomes, the somatic number, are present, as very long bands in earlier stages, and contracted to an ovoid form at the time when the nuclear membrane is dissolving and centrosomes and spindle fibres are visible.

The ovary of the adult animal, seen in cross-section (Pl. 1, Figs. 1 and 2), is nearly circular in outline and consists of an outer covering of endothelium, connecting by a short mesentery with the endothelial lining of the coelom; an oviduct — or in all probability merely a spermduct — with large deeply-staining nuclei and indistinct cell boundaries; and surrounding the spermduct, a layer of epithelium which extends out as a fold on either side like the arms of a crescent, and from the median central region of which the ova develop. Within the arms of the crescent are oocytes of various sizes (Figs. 1 and 2).

Fig. 1 shows a section of a much smaller ovary than Fig. 2, the magnification being about two and one half times as great as in Fig. 2. Sections of still younger ovaries show essentially the same conditions, with the side wings of epithelium much shorter, only very young oocytes, and in very young ovaries, no lumen in the spermduct. In Fig. 2 the largest oocyte is nearly ripe, probably within a few hours of laying. The nucleus was drawn from another section of the same egg.

Development of the ova. The youngest oocytes are always found developing from the median central portion of the layer of germinal epithelium (Fig. 1), suggesting the possibility that only that portion of the layer is the true germinal epithelium, and that the remainder, which furnishes follicle and other accessory cells, has a different origin, but this is a question which can be answered only by a study of the embryological development of the ovary. Dividing cells are found in all parts of this epithelial layer; in several cases I have found the somatic number of chromosomes, 18, in cells of the side wings, but have not been able to count them in the central region, they are always so massed together in division stages.

As a rule, the central portion of the ovary contains only a mass of very young oocytes, often 10—12 in a section, with deeply-staining large chromosomes, a very small amount of cytoplasm, and very indistinct nuclear and cell membranes. Occasionally there are found on the border of the spermduct cells with large granular nuclei and irregular, deeply-staining granules in the nuclear membrane, as shown in Fig. 3a; in the same figure are seen two ordinary epithelial cells, b, just outside of the ovarial region. Figures 4—8 show the youngest oocytes in which the chromosomes can be counted with certainty, and in such nine are present, very often in the form of loops of different length and thickness, and always with an uneven surface. These oocytes were all found in the central region of the ovary, some lying against the wall of the spermduct, others apparently detached from it. Fig. 4 shows a very common form, often longer, with very large black granules in the nuclear membrane. These granules, which are also seen in Figs. 6, 8, 9, are irregular in form, number, and location in the membrane; they become smaller and more numerous as the oocyte increases in size and evidently go to form the reticular network which is conspicuous in the nuclear membrane of all oocytes up to the time when the egg-membrane forms. A small portion of this reticulation is shown in a surface section of a nuclear membrane in Fig. 1a.

The regular arrangement of loops in such oocytes as are shown in Figs. 5 and 6, indicate the possibility that they may have begun their development after the last oogonia division without an intervening resting stage, and that the reduction in number to nine bivalent chromosomes may have recently taken place. Figs. 7 and 8 were intended to show the expanding and lengthening loops, and no attempt was made to show all of the chromosomes.

Fig. 9 shows a section of a somewhat older oocyte in about the same stage as Fig. 1b. It was just outside of the region of smallest oocytes, and was attached to one of the epithelial cells bordering on that region. In this stage, the cytoplasm stains more deeply, and the chromosomes, which have become much more irregular in outline, have begun to send out fine branches of less stainable material than the body of the chromosomes. Figs. 4—9 were all drawn with the same magnification, BANCH & LOMB, ob. $^1/_{12}$, oc. C.

As the oocyte increases in size, the nucleus becomes larger, and the chromosomes more widely separated; the fine side branches grow longer, and the body of the chromosome appears to be composed of irregular granules, variously arranged (Figs. 1c and d). It will be noticed that these oocytes are connected with the wall of the spermduct by two cells derived from the epithelial layer of the ovary. Up to this point, the cytoplasm of the oocyte stains rather deeply and shows no special structure. The four largest oocytes shown in Fig. 1 are of about the same age as the four smallest in Fig. 2.

Fig. 10 shows an older oocyte, drawn to the same scale as Fig. 2: here the cytoplasm has begun to assume the reticular character so conspicuous in the larger oocytes of Fig. 2, and globules of yolk material are seen at the periphery. The side branching of the chromosomes is somewhat coarser and more irregular than in the preceding stage, and the branches stain more deeply. In Fig. 2a, a slight thickening in the hitherto very thin membrane is perceptible on the side farthest from the spermduct; the yolk globules are much more numerous at the periphery, and a few are scattered in the cytoplasm, which is now plainly reticular; the chromosomes are somewhat shorter, very irregular in outline, and the finer less stainable side branchings have disappeared, perhaps withdrawn into the body of the chromosome.

In Fig. 2b, is shown a nearly ripe oocyte with thick membrane, evenly distributed yolk globules, and chromosomes reduced to very short and rather thick rods which are also shown in Fig. 11. In all oocytes at this stage, a large number of black granules appear near the nuclear membrane, which is much thinner than on the opposite side; this group of granules is always found between the nucleus and the spermduct. The cytoplasm at this point is of a different character and stains more deeply. There is every appearance here of an outward movement of material from the nucleus into the cytoplasm. In the stages where the greatest reduction in the size of the chromosomes occurs, the nuclear plasm] stains more deeply as though filled with fine granules of chromatin, and large granules are seen attached to the chromosomes. Fig. 12 shows a section of a nucleus containing one long slender chromosome with four granules, and fewer granules outside of the nuclear-membrane than in Fig. 11. In Fig. 15, such a chromosome and parts of two others with large granules are shown with higher magnification. Fig. 14 shows a case where all the chromosomes, slightly longer than those in Fig. 11, were grouped in the center of the nucleus in two sections, the nuclear plasm was deeply stained, and only a few granules were outside of the membrane. That these cast out granules are chromatin removed from the chromosomes during the process of their reduction in size seems evident; but whether they pass out as granules through the partly dissolved membrane, or go into solution in the nuclear-plasm, pass out, and are reformed, it is impossible to tell with certainty. The fact that one rarely finds such granules in the nucleus except in connection with the chromosomes, would favor the latter supposition, while the appearance of the group of granules in a region of deeper-staining substance, separated from the nucleus by a scarcely perceptible membrane (Fick, 1899), gives one the impression that there has been a flow of nuclear-plasm and granules out into the cytoplasm.

Figs. 13a—i give a series of chromosomes drawn with the same magnification as Fig. 2, to show the changes which occur during the reduction from the greatest length, a, in the stage shown in Fig. 10, to the shortest, i (about one eighth the length of a) as shown in Fig. 2b and Fig. 11. Throughout the series the body of the chromosome seems to contain irregular granules, closely packed together in the reduced forms, and spread apart in the longer and branched forms. The branching and reduction in size recall the figures of Rückert (1892) for ovocytes of *Pristiurus*, but the branching is much less regular. Rückert also describes a similar relation of granules or nucleoli to the chromosomes, but in *Pristiurus* the nucleoli remain within the nucleus. In *Sagitta*, there is no such doubling of the number of chromosomes as in *Pristiurus*, but the number nine is maintained without interruption from the beginning of the growth period, to the time of fertilization, the splitting of the chromosomes appearing in the first maturation division (Boveri, 1890).

Thus in *Sagitta*, there is no disappearance and reappearance of chromatin in varying forms, as described by Carnoy & Lebrun (1897, 1898, 1899) and by Fick (1893, 1899) for Amphibia; but unquestionable continuity of the reduced number of chromosomes during the whole growth period of the oocytes. The nucleoli in this case seem without doubt to be excretion or reduction products, and not the "Nucleinspeicher" or "Nucleinlaboratorium" of Fick (1899).

Method of fertilization. — The young oocytes, as they increase in size, move out from the central region of the ovary (Fig. 1b and e) and each one becomes connected with two of the epithelial cells which are just lateral to that region. One of these accessory cells remains attached to the wall of the spermduct, while the other gradually comes into a position, between it and the oocyte, and later sinks into the surface of the oocyte. The relation of the oocyte to its accessory cells in early stages is well shown in Figs. 1b, c, d, e; in later stages in Figs. 2, 16, 17, 18.

The two accessory cells soon increase in size, and are easily distinguished from the other epithelial cells by their different staining qualities, and by their larger nuclei and usually much larger nucleoli. The cell next to the oocyte always stains much more deeply in later stages than the other cell. Fig. 17 shows these cells in connection with an oocyte of about the age of that shown in Fig. 10. Within the outer cell has appeared a flask-shaped cavity with the neck penetrating the wall of the spermduct; the other cell is flattened against the end of the oocyte and is still separated from it by a distinct membrane. In Fig. 18, the development in the outer cell has gone somewhat farther; the tube has passed nearly through the spermduct wall; and at the other end of the vesicle, a second tube has penetrated the other accessory cell, which is now sunken into the surface of the oocyte. At this stage no separating membrane is evident between the inner accessory cell and the cytoplasm of the oocyte.

In Fig. 2b, the tube has extended through the wall of the spermduct, and a thick membrane has formed over the whole surface of the oocyte except the portion covered by the accessory cells, where a micropyle-like opening is formed.

Fig. 16 shows a slightly later stage more highly magnified. Here the spermatozoon has made its way from the spermduct through the tube *c*, and the opening in cell *a* into the tube which extends through cell *b*. This is the latest stage that my material contains. In all cases where the spermatozoon has entered the accessory cells, the base of the tube in the spermduct wall is much thickened and probably closed. This figure also shows the laminated structure of the egg-membrane, and its relation to the accessory cells. In some cases the egg-membrane is closely applied on all sides to the surface of the two accessory cells, as on the right-hand side of the figure; in others, one or more epithelial cells are connected with the micropyle opening, as on the left-hand side of the figure; and very often an opening is seen between the cells and the membrane, as though the egg were on the point of breaking away between the two accessory cells.

Claparède (1863) described the oocytes of *Sagitta* as having a pedicel composed of 5 or 6 cubical cells, and Grassi (1883) describes and figures a pedicel consisting of a single cell which, he says, in nearly ripe ova becomes amorphous and in some preparations seems to be perforated; but neither of these authors associated these cells with the process of fertilization, and I know of no other such case in the literature. To determine what happens between the stages shown in Figs. 2a, and 16, and the giving off of the polar bodies, as described by Boveri (1890), it only remains to kill the animals at the moment when the laying of the eggs begins, and study sections of the ripe oocytes remaining in the ovary. This I hope to do at some future time.

I have examined my sections very closely for an opening from the ovary into the oviduct, but find none. The indications are, however, that such an opening must be formed at the posterior larger end of the sperm-oviduct when the eggs are laid; and that the ova, already containing the sperm nuclei, break away from the outer accessory cells, and pass posteriorly through such an opening, and thence out into the water. If such is the case, as Hertwig thinks probable (1880), only the posterior end of the duct is properly an oviduct, and the whole anterior portion, extending the entire length of the ovary, is simply a spermduct through which the spermatozoa reach the ripe oocytes, entering through the openings and tubes prepared for them by the accessory cells.

The points of special interest in the ovogenesis of *Sagitta* are: 1) the unbroken continuity of the reduced number of chromosomes during the whole growth period of the oocytes; 2) the increase in length and the branching of the chromosomes, as the oocytes increase in size, and the very great reduction in the size of the chromosomes as the oocytes ripen; 3) the casting out from the nucleus of a large number of what appear to be chromatin granules, at about the time when the spermatozoon enters the accessory cells; 4) the connection of each oocyte with two accessory cells, within which is developed a definite path for the spermatozoon from the spermduct to the ovum.

In regard to the reduction question, my results are entirely negative: the preparations show nothing that throws any light on the manner in which the number is reduced from 18 to 9, nor do they show any splitting of the chromosomes.

As to the continuity of the chromosomes in this form, there is no doubt, unless, perchance, they should disappear when the nuclear membrane dissolves for the formation of the first polar spindle, as described by King (1902) for *Bufo lentiginosus*; but the close resemblance in size and appearance of the smallest chromosomes in my sections to the nine small chromosomes found by Boveri (1890) in the maturation divisions, leaves little doubt as to their continuity at this point.

Spermatogenesis.

General descriptions of the testes of *Sagitta* and of the circulation of masses of sperm cells in various stages of development in the posterior coelomic cavities are given by Hertwig (1880) and Grassi (1883); but the only account of the development of the spermatozoa from the detached spermatogonia is a paper by Lee (1887), whose results differ from mine on several important points.

Lee describes the formation in the testes of polyplasts similar to those in *Lumbricus*, having a blastophore without a nucleus. I find no evidence of such formation in my preparations; and the pieces which are on the point of breaking off from the testis are of the same structure throughout, simply a mass of cells with large, somewhat granular deeply-staining nuclei with one or more large nucleoli. A part of a section of such a mass of spermatogonia is shown in Pl. 2, Fig. 19, with two cells in division, others in a resting stage, and two approaching a division stage. Two types of spermatogonia divisions are found both in the testes and in the broken off portions; one in which the daughter chromosomes appear at the poles of the spindle as 18 rods, as seen in Fig. 19a and Fig. 20 in cross-section, and in longitudinal section in Figs. 21 and 22; and another type, where a much smaller number of loops, probably nine, are found at each pole as in Fig. 19b. Similar, somewhat smaller loops are shown in Fig. 23. These figures lead me to think that the so-called synapsis stage occurs in *Sagitta* at the close of the final spermatogonia division, the chromosomes uniting in pairs at the poles of the spindle. There is so much variation in the size of the spermatogonia that it is impossible to be absolutely certain that a resting stage where the chromosomes are not visible does not intervene between this union of the chromosomes and a stage like that shown in Figs. 24 and 25, where usually all the cells of a group contain nine distinct deeply-staining loops with a somewhat crenate or beaded outline. The number is easily counted in cross-section (Fig. 26). At this stage one or two large nucleoli are present, but the nuclear membrane is not easily made out. The chromatin loops which are oriented with the ends toward the center of the mass of cells, increase considerably in size and become more open and less stainable (Figs. 25 and 27). Then comes a stage in which the nuclear membrane is more evident and contains large granules, but the chromosomes are very indistinct, or in many cells cannot be seen at all (Fig. 28a); from such cells, the nine chromosomes again appear as somewhat shortened thick loops or V's (Fig. 28b), that soon become more or less scattered in the cell, which has increased somewhat in size. These loops shorten (Fig. 29) and straighten until they assume a dumb-bell form (Fig. 30), the form in which they appear in the aequatorial plate of the first spermatocyte division spindle (Fig. 31). Figs. 31—33 show different phases of the first spermatocyte division, Figs. 34—36 of the second. The two spindles are very much alike except in size, and both the cells and the chromosomes are smaller during the second division, but with some variation in size in different sections even from the same animal. The chromosomes in the equatorial plate in both cases have a dumb-bell form, and are so closely packed together that only occasionally is it possible to count them in a polar view of the equatorial plate as in Fig. 32, where nine are plainly seen. Fig. 33 shows a large nucleolus near one of the polar groups of chromosomes; often two are present and they may be found in any position with respect to the spindle. The centrosomes are very minute, and only in rare cases can be distinguished. The spindle fibres are very delicate, and no polar radiations can be seen. The fibres which connect the daughter groups of chromosomes are much more conspicuous (Figs. 33 and 36).

In the same group of cells may often be found both spermatocyte divisions and also cells like those in Fig. 37 where the chromosomes from the last division, without an intervening resting stage, are beginning to form the sperm-head; Fig. 38 shows later stages of the same process and Fig. 39 still later stages, where the spermatids increase greatly in length, and gradually change their position, the cell walls having disappeared, until all the spermatids belonging to one group of cells are arranged in two bundles, as described by Lee. My material did not prove to be favorable for the study of the formation of the middle piece or the tail of the spermatozoon. It merely shows conclusively that the sperm-head is formed directly from the nine chromosomes from the last spermatocyte division.

In the majority of cases all the cells of a group are in nearly the same stage, but there is sufficient overlapping to show the relation of the stages; for example, all the stages from Fig. 30 to Fig. 37 may be found in different parts of one large group.

As to the value of the two spermatocyte or maturation divisions, it is difficult to come to any satisfactory conclusion. The first division appears to be a reducing division in the WEISMANN sense, a separation of bivalent chromosomes into their original constituents, but who can tell what internal changes and shifting of elements may have occurred during the growth and subsequent shortning of the chromosomes? The spherical form of the daughter chromosomes of the first division makes it impossible to say whether the second division is transverse, longitudinal, or neither the one nor the other.

The principal points where my results differ from those of LEE (1887) are: 1) in regard to the number of chromosomes, which he gives as eight; 2) in the character of the second maturation division, where he figures eight chromosomes in the equatorial plate and four at each pole; 3) in the method of formation of the sperm-head.

The most striking elements among the sperm cells of *Sagitta* are the cells of the growth period preceding the two maturation divisions, where the chromosomes appear as nine large, very regular crenate loops (Figs. 24 and 25). The large number of such cells in nearly every section indicates that this period is one of considerable duration. A somewhat similar growth stage is figured by LEE (1897) in the spermatogenesis of *Helix pomatia.*

ISHIKAWA (1891) describes maturation divisions in the spermatogenesis of *Diaptomus* which bear some resemblance to those of *Sagitta.* The chromosomes have the same form and divide in the same manner in the first division, but the somatic number is present, and in the second division, though they assume the same dumb-bell form, one half of the number go to each pole.

At some future time I hope to be able to study the embryological development of the ovary of *Sagitta*, to examine the ova when they are on the point of leaving the ovary, and to do some further work on the spermatogenesis by other methods.

Zoologisches Institut Würzburg,
Germany, July 31, 1902.

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Explanation of Plates.

Plates 1—2.

The figures were all drawn with camera from sections of material fixed with sublimate acetic, cut 5—7 μ thick, and stained on the slide by the Heidenhain iron-haematoxylin method.

Plate 1.

Fig. 1. Cross-section of a young ovary, showing very young oocytes at the center, and somewhat older ones (a—e) with accessory cells. a shows a surface view of the reticular network in the nuclear membrane. Banch & Lomb, objective $^1/_5$, ocular C.

Fig. 2. Cross-section of an ovary containing nearly ripe oocytes (b) with very small chromosomes, cast out granules, thick egg-membrane, and accessory cells with tubular opening for the entrance of the spermatozoon. a is an oocyte with somewhat reduced chromosomes, yolk globules at the periphery, and membrane thickened slightly on the median side. B. & L., obj. $^1/_2$, oc. C.

Fig. 3. Three epithelium cells (a) from the region of youngest oocytes, and two cells (b) lateral to that region. B. & L., obj. $^1/_{12}$, oc. C.

Figs. 4—8. Young oocytes from the central region of the ovary. B. & L., obj. $^1/_{12}$, oc. C.

Fig. 9. Young oocyte with one accessory cell attached and chromosomes showing short side branching. B. & L., obj. $^1/_{12}$, oc. C.

Fig. 10. Section of an oocyte somewhat younger than Fig. 2a, with much branched chromosomes, and large yolk globules at the periphery. B. & L., obj. $^1/_2$, oc. C.

Fig. 11. Section of a nucleus from an oocyte in the same stage as Fig. 2b, showing three short chromosomes, a large number of cast out granules, and the adjacent nuclear-membrane very thin. B. & L., obj. $^1/_2$, oc. C.

Fig. 12. Section of a nucleus in a somewhat earlier stage than that of Fig. 11, showing a longer chromosome with granules attached,

and fewer granules outside of the nuclear-membrane. B & L., obj. $^1/_2$, oc. C.

Figs. 13a—i. A series of chromosomes taken from oocytes of different ages to show the gradual reduction in size. B. & L., obj. $^1/_2$, oc. C.

Fig. 14. Section of a nucleus in a stage between those of Figs. 11 and 12. Here the chromosomes were all in the center of the nucleus in two sections, the nuclear plasm seemed filled with minute dark granules, and only a few granules were outside. B. & L., obj. $^1/_2$, oc. C.

Fig. 15. One whole chromosome, and parts of two others, similar to Fig. 13e, showing large granules, or nucleoli. B. & L., obj. $^1/_{12}$, oc. C.

Fig. 16. Section through the two accessory cells of a nearly ripe oocyte, showing the connection with the spermduct, the spermhead in the second accessory cell, and the micropyle-like opening in the egg-membrane, where the formation of the membrane was prevented by the presence of the accessory cells. B. & L., obj. $^1/_{12}$, oc. C.

Figs. 17—18. Earlier stages showing the relation of the accessory cells to the oocyte, and the formation of the opening for the entrance of the spermatozoon.

Plate 2.

The figures in this plate were all drawn with camera, and Zeiss objective 1,5, oil-immersion; ocular 8.

Fig. 19. Section of a group of spermatocytes just breaking loose from the testis, showing resting nuclei; a cross-section (a) of the 18 rod-shaped chromosomes at one pole of a division spindle; and one cell in division (b), where the chromosomes appear in reduced number as loops at the poles of the spindle.

Fig. 20. 18 rod-shaped chromosomes at one pole of the spindle of a dividing spermatogonium.

Figs. 21—22. Longitudinal sections of spermatogonia spindles showing only a few of the chromosomes.

Fig. 23. Chromosomes in loop-form, from a stage similar to that of Fig. 1b.

Fig. 24—25. Growth stages in which nine regularly disposed, deeply-staining loop-shaped chromosomes appear.

Fig. 26. Cross-section of a cell like those of Fig. 25, showing 18 sections, two belonging to each chromosome.

Fig. 27. A later stage in which the chromosomes stain less deeply.

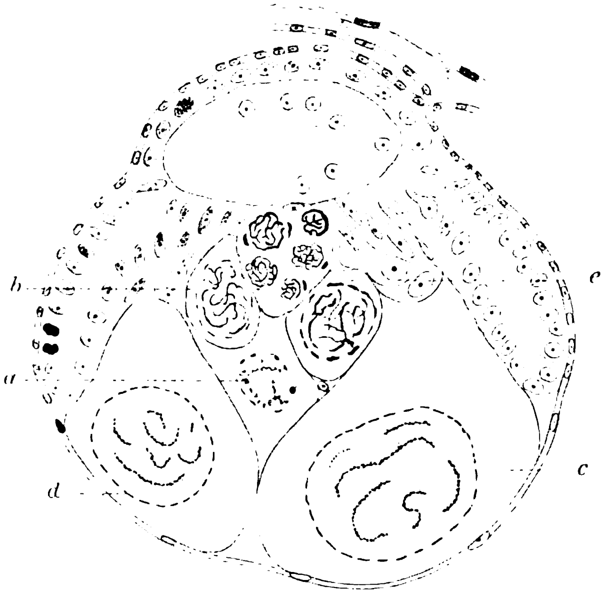
Fig. 28a. A still later growth stage in which the chromosomes are only faintly visible; b a later stage in which the chromosomes appear as shortened loops or V's, and again stain very deeply.

Figs. 29—30. Stages showing the shortening of the loops to a dumb-bell form.

Figs. 31—33. Different phases of the first maturation division; Fig. 32 polar view of the equatorial plate showing nine chromosomes.

Figs. 34—36. Different phases of the second maturation division.

Figs. 37—39. Stages in the formation of the spermhead, or spermatid from the nine chromosomes of the second maturation division.



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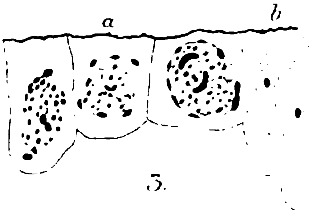

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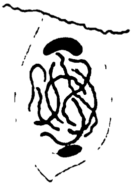

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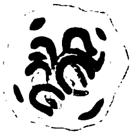

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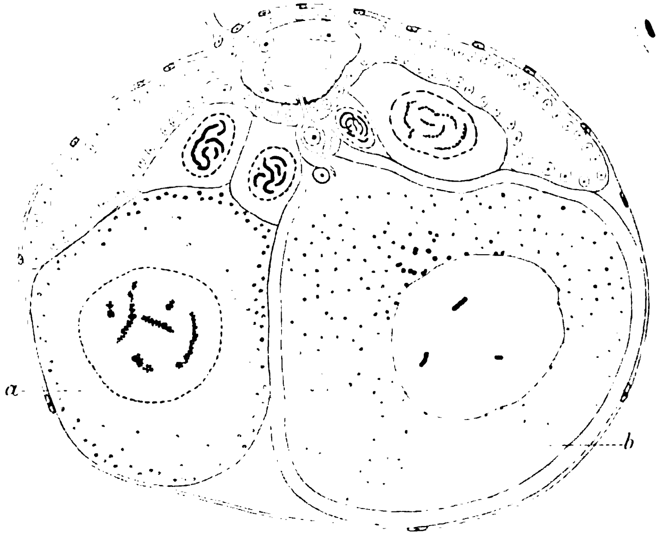

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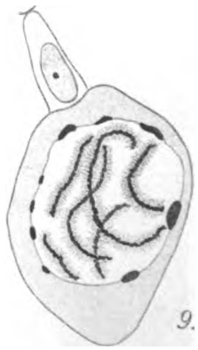

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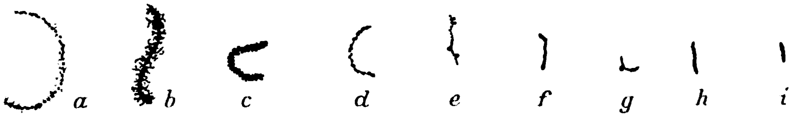

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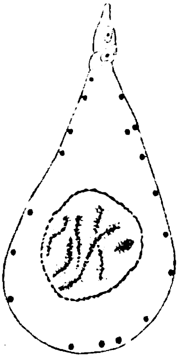

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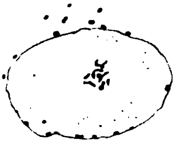

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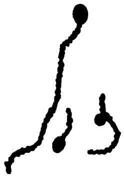

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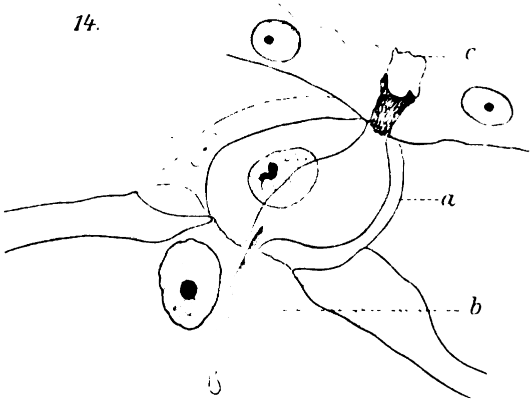

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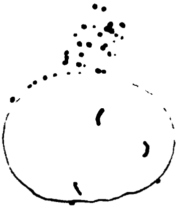

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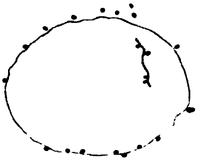

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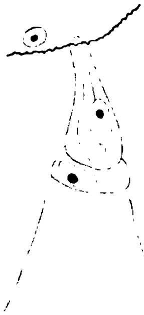

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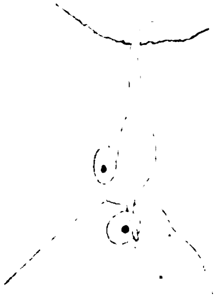

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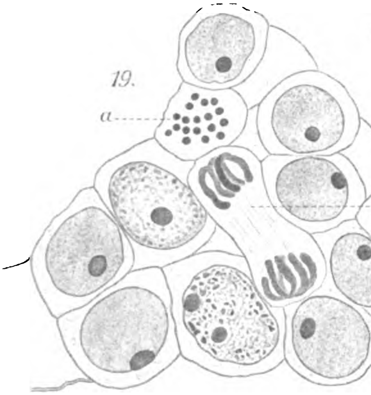
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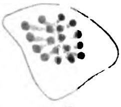
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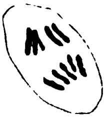
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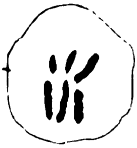
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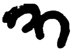
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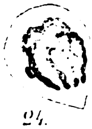
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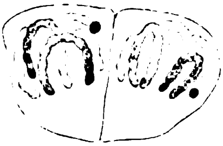
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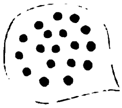
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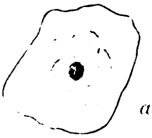
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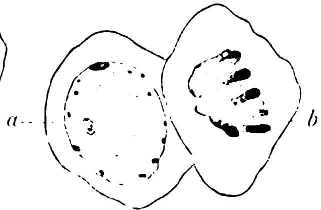
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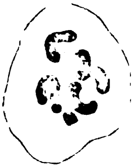
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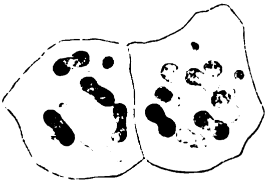
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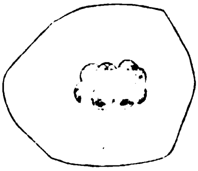
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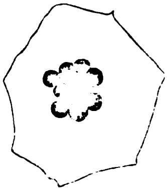
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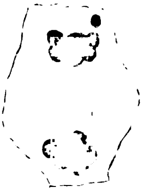
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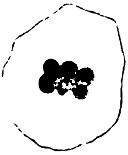
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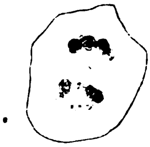
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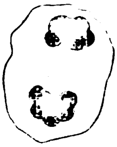
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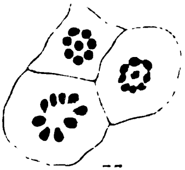
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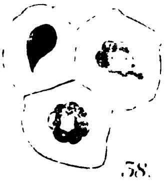
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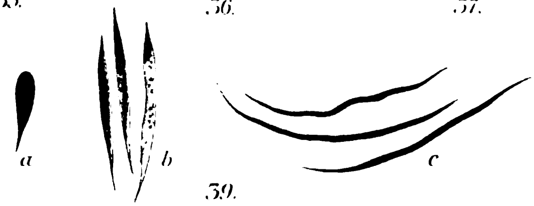
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THE RESPONSE OF THE FROG TO LIGHT.

BY ELLEN TORELLE.

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I. INTRODUCTION.

IN his study of light-response and color-sense of animals, Graber[1] states that frogs are negatively heliotropic. Loeb,[2] ~~however~~, in his paper on the extension of heliotropic phenomena in the animal kingdom, finds them ~~positively~~ negatively heliotropic.

---

[1] GRABER: Grundlinien zur Erforschung des Helligkeits und Farbensinnes der Thiere, Prag, 1884.

[2] LOEB: Der Heliotropismus der Thiere und seine Uebereinstimmung mit dem Heliotropismus der Pflanzen, 1890, p. 89.

Loeb does not give a detailed account of his observations, but Graber gives tabulated results of experiments carried out between October 10 and 20. He used a large box about 2 cm. high and divided into two compartments, one of which was dark, the other illuminated with diffuse light (daylight). Three series of experiments of ten trials each were performed with Rana esculenta, forty frogs in each trial. The frogs were placed in the boundary between the light and the dark compartment, and each trial covered a period of fifteen minutes. The totals of the results are as follows:

	I	II	III
Light . . .	133	166	174
Dark . . .	267	234	226

indicating a reaction-proportion of 15 : 10.

Loeb finds that frogs move ~~to~~ from the source of light, through whatever colored medium it be transmitted, a quantitative difference only being observable between the effects of lights of differing refrangibility. Graber had found, in three series of experiments of ten trials each, that 736 responded to the red, and 464 to the blue; the reaction-proportion being 6 : 10.

Loeb does not state whether or not he made observations with the intermediate colors, green and yellow. Graber, however, tested the response to green but not to yellow. As compared with red, the results in the two series of experiments were,

I		II	
Dark Red . . .	450	Bright green . . .	350

the reaction-proportion being 10 : 13.

Compared with blue, he found the responses to be,

I		II	
Bright green . . .	440	Dark blue . . .	280

the reaction-proportion being in this case 7 : 11. He compares the "attraction-strength"[1] of the colors, and finds them to be,

Red.	Yellow.	Green.	Blue.
1	..	0.7	0.5

In order to carry out more detailed observations than have hitherto been made on the frog, and in order to determine conclusively, if possible, its orientation to light, Dr. Morgan, to whom I am deeply indebted for kindly criticism, suggested that a series of experiments be performed to include responses of the frog to — (1) diffuse light, (2) direct light, (3) light reflected from below, (4) light transmitted from above, (5) light transmitted through a gelatine prism, (6) orientation with one eye covered, (7) orientation and reaction in high temperature and in low temperature, (8) response to monochromatic light.

---

[1] **Graber** attributed the response to differently colored lights as an exhibition of the *color-preference* of the animal.

The experiments were carried out in the Bryn Mawr biological laboratory from October to February, 1902–03. The material consisted of the two species, R. virescens virescens and R. clamata. The frogs were kept in an aquarium in the laboratory, where they seemed to remain in good condition.

In testing responses to light, two boxes were used as receptacles, the inner surfaces of which were painted a dull black, with the exception of the glass surface of one wall, through which the light was admitted. Each of the covers of the boxes contained a longitudinal slit, about an inch wide, permitting the movements of the frog to be observed without removal of the cover, a strip of black wadding which could be noiselessly lifted being laid over the slit.

At first, no time-limit was set for each trial, the frogs being kept in the box from ten to twenty minutes, or more, a varying number of frogs being used in each set of experiments. Later, all these experiments were repeated, a time-limit of ten minutes was set for each trial, and the same number of frogs was used in each set of experiments, except in cases where lack of conclusive response seemed to call for the trial of more individuals. Only one frog was used in each trial.

The results obtained fall into three divisions: Response to white light in the temperature of the laboratory; response to white light in increased and in lowered temperatures; response to monochromatic light.

II. RESPONSE TO WHITE LIGHT IN THE TEMPERATURE OF THE LABORATORY.

Diffuse light. — The response to diffuse light was first observed; a tin box, nine inches long, five inches high, and twelve inches wide, prepared as above described, served as a receptacle. In the first set of experiments, six frogs were used. Each was placed at the rear, which was the darker end of the box, with the head turned away from the source of light. Though the time of response varied with each individual, as a rule, from one-fourth of one minute to one minute sufficed for turning and for moving the twelve inches to the opposite or light end of the box. While there was usually some movement from side to side of the box at this light end, the frog remained here during the rest of the time, which in this first set of experiments was twenty minutes or more. In all cases, whether moving or resting, the median plane of the body of the frog was parallel with the incoming ray.

In a second set of observations, made a month later, five frogs were used. Each trial lasted ten minutes, and the results were substantially as before.

These results seemed to indicate that the species of frog used were positively phototactic to diffuse light (daylight), and that in diffuse daylight the orientation of the frog was such that the median plane of the body was placed parallel to the incoming ray.

Direct light. — The apparatus used in the foregoing experiment was used also in determining the response to direct light. Five frogs were used in the first set of experiments, and five in the second. The sunlight fell into one end of the box only. In each case, the response was immediate and positive. The animals moved directly to the illuminated end of the box, where they remained a variable length of time, from two to four minutes, when they moved backward, just outside the circle of bright illumination, where they remained until taken away, the median plane of the body being parallel to the incoming ray. In most cases, when the sun-illuminated area was small, the head was not turned from the light during the retreat, which was accomplished by moving first one side of the body, then the other, sidewise and backward. In other cases the frogs turned at right angles to the light, hopped outside the area of intense illumination, and orientated themselves with their heads in the direction of the incoming ray.

Since the retreat into the area of less intense illumination might have been caused by the heat of the sun's rays, the experiments were repeated, heat being cut off by placing a glass vessel with parallel sides three and one-quarter inches apart and filled with water, close to the glass end of the box containing the frog to be tested. In each case, the result was practically the same as before. When the frog was placed in the rear of the box with its head directed from the source of light, it turned, moved into the sunlight close to the glass end, where it remained a short interval, and then retreated as before, remaining in a resting position in the area of lesser illumination until removed. When placed within the area of intense illumination, with the head directed toward the source of light, it left this region as before.

Tests were made out-of-doors, with the animals unconfined and free to move into a shadow from the sunshine or *vice versa.* Ten frogs were tried. Each was placed on a glass plate, covered with a bell-jar rendered impervious to light, and carried onto the lawn near the laboratory, where it was deposited about three yards from the shadow of the building, the bell-jar removed, and the frog left on the plate with its head turned away from the sun and from the shadow of the building.

The frog at first hopped forward, then stopped, turned in the direction of the sun, and hopped well into the shadow, where it remained quietly for ten minutes. It was then moved into the sunshine, in about its former position. Again it turned and hopped into the shadow. The results were very much the same in the case of each frog tried; there was a positive and decided movement from the sunshine into the shadow.

Since the sun's rays and the shadow of the building during these experiments were in exactly the same direction from the frog, it was impossible to decide whether the movement was due to a response to the direction of the ray or toward a shadow. Therefore, later in the day, when the shadow of the building became oblique to the direction of the sun's rays, the experiments were repeated, the frog being placed in such a position that if it moved into the shadow it must hop at right angles to the direction of the rays. In each case, the results were substantially these: First, the frog turned in the direction of the (sun) ray; second, it moved quickly into the shadow by a direct path. The experiments were repeated on different bright days, but the results were always the same as regards movement from the sun-illumined area into the shadow. In some instances the frog remained in the grass; in others, it moved close to the wall of the building.

The question now arose — Does the frog recognize the shadow as an area of less intense illumination, or would it move toward or onto a black surface as well if this were placed in the sunlight?

The side of a large wooden box was covered with black cloth, and the frog placed near the black perpendicular surface. It hopped close to this, remained but a couple of minutes, then moved to the wall of the gray-colored building, where it remained at rest in the angle formed by the wall and the ground. When placed near the uncovered box (pine) on the side in full sunlight, there was no movement toward it. When the box was raised on one edge and propped, so that the other edge was about four inches from the ground, the frog moved toward the shadow thus formed, crept well under the box, placed the body between its floor and the ground, where it remained with its head directed outward.

A black cloth was fastened close to the ground in the centre of a sun-illumined area, and a frog placed near it moved onto it, crept along the edge as if seeking cover, then hopped off. A second frog also hopped onto the cloth, but almost immediately moved off. Apparently a dark surface, brightly illuminated, does not produce the effect of a shadow or of diffuse light.

Tests were also made at mid-day on a level tract of ground about two acres in extent, which contained neither trees nor any object that could cast a shadow. Six frogs were tried. When freed, each moved indifferently toward any point of the compass, but usually kept on moving in the direction in which it began to move. In several trials no movement resulted; the frog crouched low between short bunches of grass, its head held close to the ground. When dark black or dark brown screens were placed in the middle of this area, and the frogs placed within five yards of them, the movement was toward and into the shadow of the screen, where they usually remained indefinitely.

Diffuse light versus sunlight. — A tin box eighteen inches long, three inches high, and three inches wide, painted a dull black inside and with the opposite ends, consisting of glass plates, placed so that the sun's rays were transmitted through one end and diffuse light through the other, was used as a receptacle. Five frogs were tried, each being given three trials, in each of which the first position in the receptacle was changed. That is, the frog was deposited first at the end at which diffuse light was transmitted, then in the middle of the box, then near the end at which sunlight was transmitted. In each case the frog turned toward and moved to the end at which the direct ray was transmitted, but did not remain within the circle of most intense illumination. In some cases it moved to the opposite end of the box; in others, without turning, it retreated into the area of less intense illumination.

This experiment corroborates and reinforces the results obtained with diffuse light and direct light.

Light reflected from below. — In this set of experiments, a tin box, nine inches long, five inches high, and twelve inches wide, was used, all of whose surfaces were painted a dull black except the floor, which was made of window-glass. The box was supported so that the movements of the frog could be watched from below as well as from above. In the first set of experiments, five frogs were tried; in the second, fifteen. The results in both cases were the same, and differed with the amount of light (diffuse) admitted.

(*a*) When light was reflected from the whole area of the lower surface, the frog remained in normal resting position.

(*b*) When light was reflected from one-half of the lower surface, the frog hopped toward the light area.

(*c*) When the light was reflected from one-third of the surface, there was movement toward the light area, but the head was held at a greater angle to the horizontal.

In all the above trials it was found that the less the amount of light admitted, the greater the angle of the head to the horizontal plane of the floor of the box; so that, when light was reflected from the entire lower surface, a normal resting position was taken, about two-thirds of the ventral and posterior part of the body resting on the plate. When two-thirds of the lower surface of the box was covered (opaque to the light) only one-third of the ventral and posterior part of the body rested on the glass plate. In each case the frog moved from the darkened area onto or near the lighted area.

Light transmitted from above. — The box used in Experiment III was used here, the glass plate serving now as the upper side of the box. Eighteen frogs were tried; the average of results was about the same. In all cases the response to the direction of the incoming ray was immediate. The body was raised to an angle of about 45° to the horizontal. If a portion of the upper surface was covered, the frog moved to the uncovered side. Frequently, too, the frog jumped upward, toward the source of light.

Later, it was seen in experiments on five frogs, that the angle of inclination of the body varies as the distance of the frog from the upper illuminated surface. Each frog was placed in a tall glass jar which rested on a black cloth and was covered laterally by an opaque black cloth. If the entire lower and one-half of the lateral surfaces were covered, the angle of inclination of the frog's body was about 45°.

If the entire jar was covered the body was raised so that the forelegs were as nearly as possible at right angles to the horizontal bottom of the jar. This made the inclination of the body 60° or over. Frequently the frog assumed an almost erect position, by means of placing the forefeet against the side of the jar. Some of these results can be demonstrated at any time by simply placing a frog in a tin pail and covering the pail with a wire gauze. The results are valuable here, together with those of the foregoing experiments, as showing that the frog is positively phototactic to light coming from any direction.

Phototaxis in water. — Is the frog positively phototactic in water? In order to answer this question a frog was placed successively in tubes of varying diameters, the smallest being one and three-eighths inches, one end closed with wire gauze, the tube placed at angles of inclination varying from 45° to a plane parallel with the floor of the receptacle, the end covered with gauze being held near the wall of the receptacle. Light was admitted from one end only, and the tubes were completely immersed in water. Five frogs were tried, each in three trials. All moved close to the illuminated end of the tube.

Orientation. — In the first five experiments, the floor of the receptacle was bare, being kept moist by occasional rinsings with cold water. It seemed desirable to ascertain if the movements and accuracy of orientation would be affected by the presence of a bank of sand or pebbles in the box, between the source of light and the frog. Upon six inches of the central longitudinal area and across the entire width of a box nine inches wide, twelve inches long, and five inches high, a bank of sand two and one-half inches high was made, the sides of which sloped gradually toward the darker and toward the illuminated ends of the box. Twelve frogs were tried. The movements of one frog will be followed as illustrative of the response of all.

The frog was placed in the rear compartment, with its head turned from the source of light. It immediately turned around, moved to the bank, where it paused, *crawled,* not hopped, up the bank to the top, then across the plane surface to the opposite edge, where it remained one and one-half minutes, then crawled down the bank and moved close to the glass at the light end of the box.

The same frog was again placed in the rear compartment after about one and one-half inches of water had been poured into it. It swam about at first, then crawled up the bank in the direction of the light, turned again toward the water, but soon moved to the lighted edge of the bank, where it remained four minutes, when it was removed and water poured into the lighted end of the box. Within one minute the frog had crawled over the bank and into the water at the lighted end, where it remained during the rest of the experiment, or nine minutes, moving from side to side of the box with its head against the glass end.

The responses of the other eleven frogs varied somewhat with the individual, but were in the main like the one above described.

Light transmitted through a gelatine prism. — A triangular prismatic plate, three inches in diameter at the base, was made by mixing lamp-black with dissolved gelatine and allowing it to become firm. This was then placed in front of the glass end of a box with the thick end of the prism to one side, so that light of differing intensities was admitted at the same time into the box. From time to time the position of the prism was reversed. In all cases the frog hopped to the side of the box at which the most light was transmitted, *i. e.* the thin part of the prism, with the median plane of the body in the direction of the incoming ray.

The orientation of the frog with one eye covered. — The left eye was first covered with black cambric of several thicknesses, cut and sewed together so as to fit smoothly over the left portion of the head, above the nostril and anterior to the tympanum. This cap-like garment was fastened to a cambric band passed around the body just posterior to the forelegs. The frog was placed in the box used in the former experiment, with its head directed from the source of light. It immediately turned, with its right eye directed toward the source of light, *i. e.* with its body oblique to the incoming ray. The angle of deviation from the direction of the incoming ray differed in different individuals. Five frogs were tried, but in no case was the orientation that observed when both eyes were uncovered.

Next, the right eye was covered in the same way that the left one had been, and now the frog orientated itself so that the left eye was directed toward the incoming ray. The frog was then removed from the box and allowed to jump freely on the floor; the movement was toward the left, and the frog alighted on the floor on one side, in an uncertain, floundering way.

In these experiments the responses were no doubt modified by the irritation caused by the covering, of which each frog tried to rid itself. That all the movements were due to this cause cannot be concluded, for in each set, when the right or the left eyes were covered the orientation was characteristically different, as if resulting from differing causes, and not merely similar movements caused by the irritation of the covering.

Effect of prolonged light. — Does exposure to light for a prolonged period alter the response to light-stimuli?

In order to answer this question two frogs were kept confined in glass-lined boxes two and one-half inches by one and one-half inches, the ends of which were covered with wire gauze. Since the frog turns in a very small space, cords were passed through the boxes from side to side and one-half inch from the top, forming a sort of fence, which allowed space for up and down movements of the head, but not for turning around. One frog was kept in the box from 11 A. M. to 4 P. M.; the other from 8.20 A. M. to 4.20 P. M. When freed and placed in the box with one lighted end, the response was the same as before, *i. e.*, the frogs were positively phototactic and also moved close to the lighted end of the box.

The foregoing experiments seem to indicate two different kinds of response to light. One kind, the response to the direction of the rays which affect orientation, is unquestionably phototaxis; the other I shall not venture to call photopathy in the present unsettled definition of the term.[1]

III. RESPONSE TO WHITE LIGHT IN INCREASED AND IN LOWERED TEMPERATURES.

The effect of increased temperature. — The temperature of the aquarium in which the frogs were kept varied from 12° to 15° C. The temperature of the room in which the experiments were performed varied from about 18° to 20° C. In order to observe the effect of a rise in temperature on the character of the response to light, the box, before described, was placed within a large box, also having a glass end, and into which enough water could be poured to come well up the sides of the inner box. This water was heated by means of an Argand burner placed under the larger box. The temperature could be increased or kept constant as desired. A marked acceleration in time of response was noted in temperatures up to and including 25° C. The frog moved immediately and directly from the darker end of the box to the lighted end, where it remained close to the glass. Between 25° and 30° C. the frog became restless and moved about much. Above 30° C. movements toward the darker end were as frequent as those toward the lighter end, the response to light being overcome by the effect of heat.

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[1] HOLMES, S. J.: This journal, 1901, iv, p. 211; HOLT and LEE: This journal, 1901, ix, p. 460.

The effect of lowered temperature. — In observing the effect of lowered temperature upon the response to light, the same apparatus was used as described for Experiment I b, *i. e.* a small tin box, twelve inches long, nine inches wide, and five inches high, containing a glass end, within a larger box sixteen inches long, twelve inches wide, and eight inches high, also furnished with a glass end. The bottom of the smaller box was covered with a layer of sand one-half inch thick, and the box was surrounded by ice placed in the larger box. The temperature of the water in the aquarium in which the frogs were kept was 15° C., the temperature of the box was 8° C. When a frog was placed in its rear end, head turned from the light, it moved to the light end once, remained there for one-half minute, but retreated, turned away from the light, and remained in the rear of the box, either moving about, its head down as if it were trying to get under something, or quietly crouching, with the head down during the other nine and one half minutes of the experiment. When returned to the aquarium, the above movements continued in the water, the frog remaining for several minutes on the floor of the aquarium. Five frogs were tried; three of these did not leave the rear end of the box at any time.

Reaction in water to lowered temperatures. — In order to test the reaction of the frog in water to lowered temperatures, a glass jar sixteen inches by eight inches in diameter was filled with water, and set in a box containing ice, so that the lower one-third of the surface was surrounded by ice; in other respects the jar was left entirely uncovered.

When the temperature of the water in the jar was 8° C. the frog was put into it. With swimming movements it went down almost immediately, head foremost, to the bottom of the jar. With legs outspread, almost at right angles to the longitudinal axis of the body, it moved about on the bottom of the jar, from time to time repeating the movements described as taking place in Experiment II b, but rarely coming to the surface.

From Experiments I, II, III b, one is led to conclude that an increase of temperature to 30° C. lessens the time of response to light, *i. e.* accelerates the rate; that below 8° C. the frog becomes negatively phototactic, whether it is in water or on a dry surface.

Stereotropism. — If opportunity be given, does the frog burrow in sand in temperatures below 8° C., or are the movements observed stereotropic responses?

In order to answer this question, sand, to the depth of several inches, was placed in a tall glass jar, the jar being then filled with water. The sand was arranged so that its upper surface sloped from side to side. Twelve frogs were tried. When the temperature of the water in the jar became 10° C. the frogs went down and remained down, with the body flat and limbs outspread, but no attempt was made to burrow. The crouching movements, together with the passing of the head over the surface of the sand as if exercising a sense of touch, continued with a lowering of the temperature to 4° C., when they ceased. When a rock was lowered into the jar in such a way that a small space was formed between it and the wall of the jar, the frog crawled into this space and remained there. When a space was formed between the bottom of the jar and the rock, it crawled into that. This was tested several times, and was also observed when the temperature of the water in the aquarium in which the frogs were kept was lowered to 10° C. and below. When this was done, all the frogs responded, either by flattening their bodies against the stone floor, or by creeping under the rocks usually kept there. It therefore seems that the frog is stereotropic in temperatures between 10° C. and 4° C.

Effect of darkness on upward and downward movements in water. — The same jar used in the preceding experiment was used for this. The upper two-thirds of the jar including the open surface was covered with a cloth opaque to the light. With the temperature of the jar at 10° C. five frogs were tried, each being left in the jar ten minutes. They went immediately to the bottom, but rose to the top at intervals as before, and their movements seemed the same as when the jar was left uncovered. When the lower two-thirds of the jar was covered, no change was produced.

IV. RESPONSE TO MONOCHROMATIC LIGHT.

The response to monochromatic light was studied in different ways. First, glass vessels with parallel sides three and one-half inches apart were used to hold solutions of pigment recommended by Davenport.[1]

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[1] DAVENPORT: Experimental morphology, p. 157.

An alcoholic solution of fuchsin was used in testing the response to red; Lyon's blue, a concentrated solution of potassium chromate, and nickel nitrate were used for blue, yellow, and green respectively. The response to each was first separately observed.

A glass vessel containing a solution of fuchsin was placed close to the glass end of the tin box used in some of the previous experiments. This box was twelve inches long, nine inches wide, and five inches high, and the inner surfaces were painted a dull black; a slit in the cover, which was overlaid with several thicknesses of wadding, made frequent observations easy and caused little, if any, disturbance to the animal.

Red. — (*a*) When placed close to the red, the frog turned and hopped to the rear or opposite end of the box. This happened two out of seven times. Five times when so placed it turned away from the red, but remained in the front half of the box.

(*b*) When placed in the rear of the box it remained there six out of seven times. The seventh time it wandered about back and forth.

(*c*) When placed at or near the middle of the box, it was indifferent as to moving backward or forward. It usually remained about where it was placed.

Yellow. — The concentrated solution of potassium chromate was used in the same way that the fuchsin had been used. Five frogs were tried in ten trials. In each case, the frog moved to the source of the light, but soon retreated, remaining seated usually a short distance from it, indifferent as to orientation.

Green. — Four frogs were tried in twelve trials. There was much moving about, to and from the green, but in no case did the frog remain for any length of time close to the green light.

Blue. — Three frogs were tried in thirteen trials. The reaction was immediate and positive. Each frog hopped close to the glass, usually with the tip of its head against it, and frequently remained so until removed.

Response to differently colored lights admitted at opposite ends of a receptacle. — The question arose as to how the frog would respond were differently colored lights admitted into opposite ends of the receptacle. In order to answer this, a tin box eighteen inches long, three inches wide, and three inches high, whose inner surfaces were painted a dull black with the exception of the opposite ends, which consisted of glass, was used as a receptacle.

Results. — (*a*) The vessels before described were placed close to the glass ends. One was filled with a solution of fuchsin, the other with a solution of nickel nitrate. Five frogs were tried. Each moved from the red to the green or toward the green.

(*b*) When the green and the yellow lights were opposed, movement was from the yellow toward the green. The frogs usually remained a few inches from the green end of the box with heads turned toward the green light, but they were not always precisely orientated by the rays.

Five frogs were tried, each in two trials.

(*c*) Next yellow and red were used in the same way. Five frogs were tried. The movement was from red to yellow.

(*d*) When the blue light and the red light were at the opposite ends, the response was an immediate movement toward the blue. The difference between the response to the blue and the responses to the green and yellow is very marked. Blue not only effects an immediate response, but the frog remains close to the glass end where the blue solution is placed, frequently with its head against the glass and its median longitudinal axis parallel to the incoming ray.

TABLE I.

No. of frog.	Time at blue end.	Time at red end.	Position of frog at beginning of experiment.
1	8 min.	2 min.	Head turned toward red and in red end of box.
2	10 "	0 "	Same as 1.
3	10 "	0 "	Same as 1.
4	0 "	10 "	Same as 1.
5	0 "	10 "	Same as 1.
6	8 "	2 "	Same as 1.

Unequal amounts of light transmitted through the red and through the blue media. — Other tests were made with a greater amount of light transmitted through the red than through the blue medium. A vessel with parallel sides three and one half inches apart was used for the blue solution, one with its parallel sides one and one half inches apart being used for the red. In order to be able to make more accurate comparisons, it was thought best to note the time during which the frog in each trial remained with its head directed toward one or the other light, a ten-minute limit for each trial being taken. Since the responses to green and to yellow had seemed conclusive, and since previous observers differed as to the response to red and to blue, attention was confined to testing the response to these colors.

The results when red and blue were opposed are shown in Table I, showing a reaction-proportion of 4 : 2 in favor of the blue, even when more light was transmitted through the red medium.

Response to a red and to a blue background.— One-half of the inner surface of a tin box twelve inches long, nine inches wide, and five inches high was covered transversely with blue, one-half with red cheese-cloth. White light was admitted through the glass, at the end, which was covered with blue. The results are shown in Table II:

TABLE II.

No. of frog.	Time at blue end.	Time at red end.	Position of frog at the beginning of the experiment.
1	7 min.	3 min.	Head turned from light in the rear of red compartment.
2	8 "	2 "	Head turned from light in the middle of red compartment.
3	8½ "	1½ "	Head turned from light in the rear of red compartment.
4	6 "	4 "	Same as 3.
5	5 "	5 "	Same as 3.

Then the strips were reversed, white light being admitted through the glass at the end, which was covered with red. The results are embodied in Table III.

TABLE III.

No. of frog.	Time at blue end.	Time at red end.	Position of frog at beginning of the experiment.
1	10 min.	0 min.	Head turned from light and in rear of blue compartment.
2	10 "	0 "	Same as 1.
3	8 "	2 "	Same as 1.
4	9¾ "	¼ "	Same as 1.
5	9 "	1 "	In the middle of the red area, head turned toward the light.

The box was lined with the strips laid lengthwise, the white light admitted equally on both; the position of the frog at the beginning of each trial, together with the results, being indicated in Table IV.

TABLE IV.

No. of frog.	Time at blue end.	Time at red end.	Position of frog at beginning of experiment.
1	9½ min.	½ min.	In rear end of red compartment, head turned from light.
2	9 "	1 "	Same as 1.
3	6 "	4 "	Same as 1.
4	9 "	1 "	In rear of blue compartment, head turned from light.
5	10 "	0 "	Same as 4.

Red and blue solutions were placed in front of the red and blue-lined sides of the box respectively, with results shown in Table V.

TABLE V.

No. of frog.	Time at blue end.	Time at red end.	Position of frog when experiment began.
1	10 min.	0 min.	In rear of red compartment, head turned toward the light.
2	5 "	5 "	In rear of red compartment, head turned from the light.
3	7 "	3 "	In middle of blue compartment, head at right angles to incoming light.
4	10 "	0 "	In rear of blue compartment, head toward light.
5	10 "	0 "	In rear of blue compartment, head turned from light.

The results of the experiments with the colored cloths did not seem convincing, for the cloth offered an absorptive surface to the skin of the frog, and the dyes as well might have vitiating influences. So the external surfaces of three rectangular glass vessels were painted in the one case blue and red, transversely applied; in the other, the position of the colors was reversed; in the third, the paint was longitudinally applied.

The blue corresponded to the tube paint known as new blue; the red, to vermilion. White light was transmitted at the open end through an ordinary window-pane.

(*a*) The vessel on which the paints were longitudinally applied was first used.

TABLE VI.

No. of frog.	Time at blue end.	Time at red end.	Position of frog at beginning of experiment.
1	10 min.	0 min.	In rear of red compartment.
2	8 "	2 "	In rear of red compartment.
3	3½ "	6½ "	In rear of blue compartment.
4	10 "	0 "	In middle of blue compartment.
5	10 "	0 "	In middle of red compartment.
6	10 "	0 "	In rear of blue compartment.
7	8 "	2 "	In middle of red compartment.
8	0 "	10 "	In rear of red compartment.
9	10 "	0 "	In rear of blue compartment.
10	9½ "	½ "	In ant. end of red compartment.
11	8 "	2 "	In ant. end of red compartment.
12	9 "	1 "	In rear end of red compartment.
13	10 "	0 "	In rear end of blue compartment.

The above table (Table VI) shows a response of eleven out of thirteen to blue. The response to red may be accounted for by fear, or by sluggishness.

(*b*) Next the vessels to which the colors had been transversely applied were used. The frog was first placed in the vessel in which the red was next to the white light. It was watched for ten minutes; then this same frog was put into the aquarium in which the blue was next to the white light and again watched ten minutes. The frog was always placed in the same relative positions in the two vessels and the positions differed for each experiment.

When red was next to the white light the responses were those indicated in Table VII:

TABLE VII.

No. of frog.	Time at blue end.	Time at red end.	Position of frog when experiment began.
1	10 min.	0	In right-hand, rear corner of blue. (Moved to red boundary and stopped.)
2	10 "	0	In anterior part of blue compartment. (Moved to red boundary and stopped.) Remained in blue compartment.
3	8 "	2	In middle of red compartment.
4	9½ "	½	In rear of middle part of blue compartment.
5	10 "	0	In rear of left-hand corner of blue compartment.
6	8 "	2	In middle of red compartment.

When blue was next to the white light the frogs responded as shown in Table VIII:

TABLE VIII.

No. of frog.	Time on blue.	Time on red.	Position of frog at beginning of experiment.
1	0 min.	10 min.	In middle of red compartment.
2	10 "	0 "	In middle of blue compartment.
3	7 "	3 "	In middle of blue compartment.
4	10 "	0 "	In rear of red compartment.
5	0 "	10 "	In rear of red compartment.
6	10 "	0 "	In middle of blue compartment.

A constancy in the response of the same frog, in vessels in which the colors are reversed, is here observed, except in frogs 1 and 5 of the last set. The first I can account for, as the glass plate at the open end fell during the experiment and frightened the frog into retreat; the case of the fifth I cannot account for except that its condition was rather sluggish.

A week later the same experiments were repeated with the following results:

When red is next to the white light the following results were obtained:

TABLE IX.

No. of frog.	Time on blue.	Time on red.	Position of frog at beginning of experiment.
1	10 min.	0 min.	In rear of blue compartment.
2	9½ "	½ "	In rear of blue compartment.
3	9½ "	½ "	In middle of red compartment.

When blue is next to the white light the results are those indicated in Table X:

TABLE X.

No. of frog.	Time on blue.	Time on red.	Position of frog at beginning of experiment.
1	8 min.	2 min.	In rear of red compartment.
2	10 "	0 "	In rear of red compartment.
3	2 "	8 "	In middle of blue compartment.

The experiments were repeated, using other frogs, and changing the time of each trial from ten to twenty minutes. When red is next to the source of light the behavior of the frog is that indicated in Table XI.

TABLE XI.

No. of frog.	Time on blue.	Time on red.	Position of frog at beginning of experiment.
1	20 mins.	0 mins.	In middle-left of red compartment.
2	20 "	0 "	In middle-left of red compartment.
3	2 "	18 "	In middle-left of red compartment.

When blue was next to the source of light the frogs responded as indicated in Table XII:

TABLE XII.

No. of frog.	Time on blue.	Time on red.	Position of frog at beginning of experiment.
1	16 min.	4 min.	In middle of blue compartment.
2	9 "	11 "	In middle-left of blue compartment.
3	12 "	8 "	In middle of blue compartment.

Calculating in minutes the response to a red or to a blue background, when white light is admitted, the response to the blue was three times greater than that to the red, the actual number of minutes on the blue being 479; on the red, 159.

Response when the entire environment is one-half blue and one-half red. — A glass plate, one-half of which was painted red, the other one-half blue, was placed before the opening of the vessel[1] so that the red of the plate was adjacent to the red of the vessel, and the blue of the plate adjacent to the blue of the aquarium. The trials were of twenty minutes' duration and gave the following results:

TABLE XIII.

No. of frog.	Time on blue.	Time on red.	Position of frog when placed in the vessel.
1	16 min.	4 min.	In rear of red compartment.
2	20 "	0 "	In rear of blue compartment.
3	17 "	3 "	In middle of red compartment.
4	20 "	0 "	In middle of blue compartment.
5	20 "	0 "	In front end of blue compartment.
6	19 "	1 "	In front end of red compartment.

In this set of experiments the reaction-proportion is as 14 : 1 in favor of the blue.

Response when white light is admitted at opposite ends of a receptacle, one-half of the surface of which is painted red, the other half blue. — A tin box eighteen inches long, three inches wide, and three inches high, and containing opposite glass ends (three inches by three inches), was laid off into equal compartments and its inner walls painted blue and red. In six trials the same frog was used, being put in the same relative position but upon a different color in consecutive experiments. In eleven trials the colors were reversed after each experiment, the frog being placed now on red, now on blue, and again on the boundary between the two. The results of the eleven trials are shown in the following table:

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[1] In which the strips of red and blue ran lengthwise.

TABLE XIV.

No. of frog.	Time on blue.	Time on red.	Position of frog at beginning of experiment.
1	10 min.	0 min.	In rear of red compartment.
2	10 "	0 "	In front end of blue compartment.
3	10 "	0 "	In middle of blue compartment.
4	10 "	0 "	In rear of blue compartment.
5	9 "	1 "	In front end of red compartment.
6	9 "	1 "	In middle of red compartment.
7	8 "	2 "	In rear of red compartment.
8	7½ "	2½ "	In front end of red compartment.
9	6½ "	3½ "	In middle of red compartment.
10	6 "	4 "	In rear of red compartment.
11	9½ "	½ "	In rear of blue compartment.

The results in the former case show a greater length of time on the blue in the case of all except the second frog, which, as indicated in Table XV, remained in the red compartment for ten minutes when placed in the red compartment.

The results obtained in response to monochromatic light seem to illustrate Loeb's theory "that the more refrangible rays are extraordinarily more active than the less refrangible, which occasionally remain almost ineffective."[1] According to Abelsdorff,[2] red rays affect the pupils of the eyes of some animals like darkness.

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[1] LOEB, J.: Der Heliotropismus der Thiere, p. 20.

[2] ABELSDORFF, G.: Archiv für Physiologie, 1900, p. 561.

Graber's result on the frog can be explained only on the ground of a confusion arising as a result of using so many frogs (forty) at the same time in one receptacle.

TABLE XV.

No. of frog.	No. of trials.	Time on blue.	Time on red.	Position of frog at the beginning of the experiment.
1	1	10 min.	0 min.	At glass end of blue compartment.
	2	10 "	0 "	At glass end of blue compartment.
2	1	9 "	1 "	At glass end of red compartment.
	2	0 "	10 "	At glass end of red compartment.
3	1	8 "	2 "	At glass end of blue compartment.
	2	9½ "	½ "	At glass end of blue compartment.

CONCLUSIONS.

1. At the usual temperature of the laboratory, between 16° and 21° C., Rana virescens virescens and R. clamata are positively phototactic.
2. They respond to light coming from above and from below, as well as from the side.
3. They respond differently to different intensities of light. They move out of the sunlight into the shadow, even when by so doing the movement is away from, or at right angles to, the direction of the ray.
4. When one eye is covered, the body is placed with its median plane oblique to the ray.
5. When a bank of sand is interposed between the frogs and the light, they crawl over this and move to the source of light.
6. A rise in the temperature to 30° C. accelerates the rate of the positive response. A lowering of the temperature to 10° C. produces movements away from the light.
7. When placed in water the temperature of which is lowered to 10° C., the frogs swim downward: (*a*) In an uncovered glass vessel; (*b*) In a vessel, the upper two-thirds of which has been darkened; (*c*) In a vessel the lower two-thirds of which has been darkened.

8. The frogs turn away from red light and move toward blue light. They move toward green and toward yellow light, but are not definitely orientated by either.

9. When red light is admitted at one end and green light at the other end of a receptacle, the frogs move from the red to or toward the green. When red and yellow lights are opposed in the same way, movement is from the red to the yellow. When red and blue are opposed, movement is immediately toward the blue.

10. When white light is admitted at one end of a receptacle, and the frogs are given a choice of a red or of a blue environment, they move, in most cases, into the blue, and remain in it longer than they do in the red.

11. When one-half of the entire receptacle is blue and the other half is red (no white light being admitted), movement is from the red to the blue.

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THE EFFECTS OF HEAT ON THE DEVELOPMENT OF THE TOAD'S EGG.

HELEN DEAN KING.

An extended series of experiments made by Hertwig (1–4) prove that the maximum temperature at which the eggs of the frog will develop normally differs for different species. His experiments also show that eggs in the cleavage stages can withstand a higher temperature than can unsegmented eggs. These results have a bearing on the general problem of adaptation; for it may be possible to show, after more species have been studied, that the maximum temperature which the eggs of amphibians can endure without injury and also the temperature most favorable for their development depend, to a certain extent at least, on the time of year at which the eggs are deposited.

MATERIAL AND METHOD.

The eggs of the common toad, *Bufo lentiginosus*, were used in making all of the experiments recorded in the present paper. After natural fertilization, the eggs were brought into the laboratory where the temperature varied from 18 to 21° C. Control sets of eggs from each lot used for the experiments, developing at the room temperature, all became perfectly normal embryos, and some of them were kept until metamorphosis.

In making the experiments, small dishes containing about 80 c.c. of spring water were placed in the drying chamber of a large water-bath, and after the water had become heated, from 50 to 75 eggs were quickly transferred into it and left a given length of time. The temperature to which the eggs were being subjected could readily be told from a thermometer that projected into the chamber through a small opening in the top. Great care was taken to keep the temperature of the chamber as constant as possible during the course of the experiments, and in no case did it vary more than two degrees. After the eggs were removed from the chamber, they were put into fresh water at room temperature and their later development compared with that of the eggs in the control set.

II. EXPERIMENTS ON UNSEGMENTED EGGS.

Experiment 1. — On April 16, twenty-five unsegmented eggs were subjected to a temperature of 28–30° C. for two and one-half hours. When removed from the chamber, all of the eggs were in the 16-cell stage, while in the control set, developing at room temperature, the eggs had only reached the 4–8-cell stage. The immediate effect of the higher temperature, therefore, was to increase the rate of development. This result agrees fully with that obtained by Hertwig in many of his temperature experiments on the frog's egg. The later development of the eggs in this series appeared to be perfectly normal, and it took place at about the same rate as in the eggs of the control set.

Experiment 2. — A number of eggs that had not yet segmented were put into water at a temperature of 30–32° C. on April 17. Part of the eggs were removed at the expiration of three quarters of an hour, and when examined they were all found to be segmenting. In a few cases the first cleavage plane had nearly cut through the yolk portion of the egg and the second furrow was appearing. In the control set of eggs, the first cleavage plane was just coming in at this time, so that, in this experiment also, the early development became more rapid as an immediate result of exposing the eggs to a higher temperature. All of these eggs developed into normal embryos.

Some of the eggs of the above lot remained in the heated chamber for one hour. The second cleavage plane had appeared in all of the eggs when they were removed to room temperature. Later segmentation was normal, and on the following day the dorsal lip of the blastopore appeared in all of the eggs at about the same time that it formed in the eggs of the control set. On April 19, many of the eggs were dead; some were in the early gastrula stages, and some showed traces of the medullary folds. Of the seven embryos alive on April 20, three were abnormal, having a large yolk plug exposed at the posterior end of the body; the other four embryos were normal and were kept for several weeks.

The remaining eggs of this lot were kept at the temperature of 30–32° C. for one and one-half hours. At the end of this time they were in the 16-cell stage, while the eggs of the control set were only in the 2–4-cell stage. Later segmentation of these eggs seemed to be normal, and on April 18 the dorsal lip of the blastopore appeared in a very few of them. On the morning of April 19 most of the eggs were dead, and not one of them, when examined, was found to have gastrulated. In the eggs still living the blastopore was closing in, but development was much slower than that of the eggs of the control set in which, at this time, the blastopore had already closed and the medullary folds were forming. All of the eggs were dead on the morning of April 20, and in no case was gastrulation entirely completed.

In these last two lots of eggs the injurious effects of heat were not apparent during the segmentation stages and only manifested themselves when the eggs were ready to gastrulate. Early development was accelerated; but later development lagged behind, or, at most, was equal to that of the eggs in the control set.

Experiment 3. — A number of unsegmented eggs were exposed to a temperature of 32° C. for two hours on April 22, and when removed they were in the 16-cell stage. In this lot of eggs the later cleavage was very abnormal as the upper hemisphere divided into a number of small cells, while the lower part of the egg segmented only a few times and, consequently, was composed of a small number of very large cells. Cleavage lines were very distinct in the upper part of the egg; but it was almost impossible to make out the boundaries of the yolk cells. None of the eggs in this set gastrulated and all of them were dead by April 24.

Experiment 4. — On the morning of April 16, a small lot of eggs was subjected to a temperature of 32–33° C. for one-half of an hour. The eggs had not segmented when they were put into cooler water, but in every case the first furrow appeared in about fifteen minutes. In the control set, the first cleavage plane came in about half an hour later than it did in the eggs used for the experiment. All of the eggs of this set developed normally, and sections made of later embryos showed them to be no different from the embryos of the control set.

Experiment 5.— A bunch of about seventy-five unsegmented eggs was put into water heated to a temperature of 34–35° C. on April 16. Part of the eggs were removed at the end of half an hour and a few of them at once began to segment. None of the cleavage planes, with the exception of the first, came in normally, and in no case did any of them cut through the entire egg. Part of a section of one of these eggs is shown in Fig. 1. All of the cleavage planes are seen to be parallel and to extend but a short distance through the upper hemisphere of the egg. Development did not progress beyond this stage in any case, and the majority of the eggs never segmented although they appeared to be living several hours after they were brought into room temperature.

Some of the eggs of the above lot remained at the temperature of 34–35° C. for one hour. When put into cooler water and examined, a slight depression was found in the center of the upper hemisphere of a few of the eggs as if the first cleavage plane was about to appear in its normal position. This appearance, however, proved to be only a wrinkling of the surface as none of the eggs, when sectioned, showed any true cleavage planes.

The above experiments show that the unsegmented eggs of the toad can withstand a temperature of 32–33° C. for one-half of an hour and develop normally, while an exposure to this temperature for a longer period is very injurious and only a small per cent. of the eggs produced normal tadpoles. Exposure to a temperature of 34°, even for a short time, injures the eggs beyond the possibility of a recovery. The maximum temperature that the unsegmented egg can endure without injury is, therefore, 33° C. The optimum temperature, a term defined by Hertwig (3) as, "Die Temperatur bei welcher sich der Entwicklungsprocess bei allen Eiren mit der grössten Beschleunigung ohne eine auffällige Störung und Abweichung von der Norm vollzieht," for this egg is probably not far from 28° C., judging from the results obtained in experiments 1 and 2. In all cases in which the heat did not kill the eggs, development was accelerated at first, apparently with no injurious effects on the egg. In later stages,

1

2

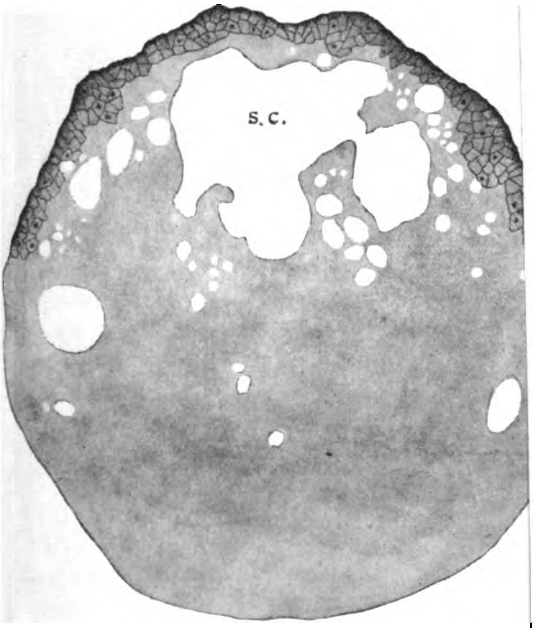

FIG. 1. Part of a section of an egg that was subjected to a

FIG. 2. A section of an egg that was exposed to a t
in the two-cell stage. *S. C.*, segmentation cavity.

FIG. 3. A section of an egg that was subjected to a tem
appeared.

FIG. 4. A section of an egg that was subjected to a tem
32–64-cell stage of development. *Bl.* blastopore.

owever, the eggs of the control sets appeared to be fully as far dvanced in development as were the eggs that had been subjected to a higher temperature. Increase in the rate of development is, therefore, but the immediate effect of heat, and after the eggs are brought into a lower temperature they develop at the same, or a lower rate, than the eggs of the control set.

III. Experiments on Eggs in Early Cleavage Stages.

Experiment 6. — On April 17, a lot of about fifty eggs in the 2-cell stage of development was exposed to a temperature of 31–33° C. At the end of one and one-half hours, part of the eggs were removed. They were then in the 8–16-cell stage. The later development of these eggs was perfectly normal in every respect.

The rest of the eggs of this lot remained in the heated chamber for two hours. All of these eggs developed normally during the early cleavage and gastrulation stages; but later a few embryos were found with shortened medullary folds and a large yolk plug at the posterior end of the body. This form of abnormality is very common among embryos that have been injured by exposure to heat.

Experiment 7. — As the first cleavage plane was appearing, a lot of about fifty eggs was subjected to a temperature of 35–36° C. for three quarters of an hour. All of the eggs were segmenting in a very abnormal manner when they were transferred into water at the room temperature, and none of them ever gastrulated. Fig. 2 shows a median section through one of these eggs. With the exception of the layer of small cells bordering the outer surface of the upper hemisphere, the entire substance of the egg is seen to be unsegmented and to have a number of different sized vacuoles scattered through it. A large, irregularly shaped cavity fills the greater part of the upper hemisphere of the egg. This cavity is much larger than the segmentation cavity in a normally segmenting egg, and it appears to be formed of the true segmentation cavity and several large vacuoles which have come to open into it.

Experiment 8. — On April 22, a lot of eggs in the 2-cell stage was exposed to a temperature of 35–36° C. for one hour. When removed from the chamber the eggs were in the 8-cell stage, but development stopped at this point and all of the eggs were dead inside of twenty-four hours.

Experiment 9. — In this experiment, eggs in the 2- and in the 4-cell stages of development, were subjected to a temperature of 33–35° C. for a period of two hours. At the end of this time the eggs were segmenting very irregularly in the upper hemisphere and no cleavage planes were visible in the yolk portion of the egg. A section through one of these eggs (Fig. 3) shows the entire upper hemisphere divided into a mass of small cells containing a considerable amount of pigment which is, for the most part, collected in the middle of the cell around the nucleus. The first cleavage plane has cut only partially through the yolk portion of the egg, as its progress was evidently stopped at the beginning of the experiment. There are no nuclei in the yolk portion of the egg, and the many vacuoles show the injurious effects of the heat. The mass of small cells in the upper hemisphere forms a sort of cap on the unsegmented yolk and make it appear as if the segmentation of the egg was meroblastic. This same sort of abnormal cleavage has also been obtained by Hertwig (1, 2).

According to the experiments in this series, eggs in the early cleavage stages can endure exposure to a temperature of 31–33° C. for a longer period than can the unsegmented egg; yet they are permanently injured by even a short immersion in water at a temperature of 35°. The maximum temperature for these eggs, therefore, is not greater than that for the unsegmented egg. Hertwig (4) has found that the maximum temperature for the eggs of *Rana fusca* in the 8-cell stage of development is 26–28°, which is 3–4° higher than that for the unsegmented egg.

IV. Experiments on Eggs in Late Segmentation and Early Gastrula Stages.

Experiment 10. — On April 18, fifty eggs in the 32–64-cell stage of development were kept at a temperature of 31–33° C. for two hours. Subsequently all of the eggs developed into normal embryos and at about the same rate as did the eggs of the control set.

Experiment 11. — Another set of fifty eggs from the same bunch as the eggs used in experiment 10, was subjected to a temperature of 31–33° C. for three hours. The late segmentation and early gastrulation stages of all of these eggs seemed to be perfectly normal. Two days after the experiment was made, 38 of the eggs were dead, the blastopore not having closed in any case. Of the remaining eggs four only were normal, the rest had a large yolk plug at the posterior end of the body.

Experiment 12. — Twenty-five eggs from the same lot as those used in the two preceding experiments remained in water at a temperature of 31–33° C. for three and one-half hours. Fifteen of the eggs died in the blastula stage. The blastopore appeared in the other ten eggs; but in many cases it was in an unusual position at the equator of the egg. When the dorsal lip of the blastopore was forming in these eggs, the circular blastopore was already beginning to close in the control set of eggs, therefore, in this instance, the heat retarded instead of increased the rate of development of the eggs. In none of the eggs of this set did the blastopore ever become circular, and all of the eggs were dead two days after the experiment was made.

Fig. 4 shows a section of one of these eggs preserved when the blastopore appeared in surface view as a short, straight line at the equatorial zone. The dorsal lip of the blastopore rarely, if ever, comes in as high up as the equator in eggs that are developing normally ; but it sometimes occupies an unusual position in eggs that have been subjected to abnormal conditions. Morgan (5) has found the blastoporic rim above the equatorial zone in eggs of *Rana palustris* that have been subjected to intense cold. In Fig. 4 the archenteron appears as a shallow depression with its dorsal wall formed of heavily pigmented cells as is normally the case. The inner end of the archenteron, instead of turning up towards the black pole as it would do in a normal egg, here projects downward towards the yolk pole. The most interesting fact shown by the section is that the normal position of the large and of the small cells of the egg is completely reversed. In normally gastrulating eggs, the roof of the segmentation cavity is formed of two to three layers of small, pigmented cells, while the ventral wall is composed entirely of large yolk cells that contain little, if any, pigment. In this egg, however, the upper wall of the segmentation cavity is made up of a single layer of heavily pigmented cells which are fully as large as any other cells in the egg. Below the segmentation cavity, a portion of the yolk is divided into a number of small cells, many of which contain pigment massed around the nucleus. Some of these cells are rounded and seem to lie free in the segmentation cavity, an appearance also noted by Hertwig (4) in eggs of *Rana fusca* that were exposed to a temperature of 29–35° C. after having reached about the 100-cell-stage of development.

Morgan has also noted the relatively large size of the cells in the upper hemisphere of gastrulating eggs of *Rana palustris* that had been subjected to cold. He suggests that this increase in the size of the cells "may be due in part to the absorption of water by the individual cells," and he adds that, "even if this is the case the cells are fewer in number than in a normal egg beginning to gastrulate." In the figure shown by Morgan, the cells of the lower hemisphere are all considerably larger than those of the upper hemisphere; the egg, therefore, must have been much more normal than the one from which Fig. 4 was drawn.

It is evident, in the case of the egg shown in Fig. 4, that the increased temperature did not injure the yolk region or retard its development as is usually the case in these experiments; on the contrary, it is the segmentation of the upper hemisphere that has been delayed, while the segmentation of the lower portion of the egg has continued. No egg in this set of experiments developed much beyond the stage represented by Fig. 4, and each of the ten eggs that were sectioned showed abnormalities of the same general character.

Experiment 13. — On April 26, about seventy-five eggs in the late blastula stage were subjected to a temperature of 33–35° C. A part of the eggs were removed at the end of one and one-half hours and they all developed into normal embryos.

A second portion of the eggs was exposed to this temperature for two and one-half hours. All of these eggs developed into normal embryos, although somewhat more slowly than did those of the control set.

A third part of the eggs remained at the temperature of 33–35° for three and one-half hours. These eggs were all dead when removed from the influence of the heat.

Experiment 14. — A number of eggs in the blastula stage were exposed to a temperature of 36–37° C. on April 26. Some of the eggs were removed from the chamber at the end of one-half of an hour. The eggs did not appear to be injured in any way by the experiment and all developed normally.

A second portion of the eggs from the above lot remained at this temperature of 36–37° C. for three quarters of an hour. All of the eggs gastrulated normally, but about half of them died before the blastopore closed. When sectioned these eggs showed no abnormalities. The rest of the eggs became normal embryos, although developing very slowly. The medullary folds had closed in the eggs of the control set when they were only beginning to unite in the eggs that had been subjected to the increased temperature.

The remaining eggs of this lot were removed to room temperature at the end of one hour. Although the eggs did not appear to be dead when they were examined, they did not gastrulate and none of them were alive the day following the experiment.

Experiment 15. — Twenty eggs in late segmentation stages were subjected to a temperature of 40–42° C. for one quarter of an hour. Development was at once stopped by the heat, and all of the eggs were killed.

Experiment 16. — When the dorsal lip of the blastopore was just appearing, a lot of about twenty eggs was put into water at a temperature of 33–35° C. and left there for three hours. All of the eggs continued to develop somewhat more slowly than the eggs of the control set and all became normal embryos.

Experiment 17. — On April 24, a lot of eggs in early gastrulation stages was kept at a temperature of 35–37° C. for one hour. In all of the eggs the lateral and ventral lips of the blastopore formed in the normal manner, but development stopped at this point and the eggs died. No abnormalities were detected when sections were made of several of these eggs.

Experiment 18. — Eggs in early gastrulation stages were exposed to a temperature of 37–38° C. on April 24. A part of the eggs were removed at the end of one quarter of an hour. None of these eggs seemed to be injured in any way by the high degree of heat to which they had been subjected and all developed, somewhat slowly, into normal embryos. The rest of the eggs in this lot remained at the temperature of 37–38° C. for one hour. They were all dead when removed to room temperature.

The results of the experiments in this series show that eggs in the 32–64-cell stage cannot withstand a temperature of 31–33° C. for a much longer period than can eggs that have just begun to segment. The maximum temperature to which eggs can be subjected without injury is practically the same for unsegmented eggs and for those in early cleavage stages, although eggs in the later stages can remain at this temperature for a somewhat longer period and still develop normally.

Eggs in late cleavage stages have a much greater power to withstand high temperature than have eggs in the earlier stages of development, as they will develop normally after exposure to a temperature of 36–37° C. for one-half of an hour. The maximum degree of heat that can be endured without injury is still higher for eggs in the gastrula stages, as they become normal embryos after being subjected to a temperature of 37–38° C. for one quarter of an hour.

The experiments described above are summarized in the following table. The number of the experiment is given in the first column; the condition of the eggs when the experiment was begun in the second column; the temperature to which the eggs were subjected in the third column; followed in the next two columns by the duration of the experiment and a brief statement of the results.

The results of these experiments are very similar to those obtained by Hertwig (1–4) in his study of the effects of heat on the development of the eggs of various species of frogs; and the abnormalities produced resemble, in many respects, those which Hertwig has described and figured. When the unsegmented eggs of *Bufo lentiginosus* are subjected to a temperature that

TABLE I.

No. of Exp.	Condition.	Temperature.	Time.	Result.
1	unsegmented.	28–30° C.	2½ hrs.	Normal development.
2	“	30–32	¾ “	Normal development.
2	“	“	1 “	Four eggs developed normally; the rest died or became abnormal.
2	“	“	2½ “	Most of the eggs died in the blastula stage; a few gastrulated but did not develop further.
3	“	32	2 “	All died in the blastula stage.
4	“	32–33	½ “	Normal development.
5	“	34–35	½ “	Irregular cleavage, no gastrulation.
5	“	“	1 “	Eggs killed.
6	2 cell.	31–33	1½ “	Normal development.
6	“	“	2 “	Most of the eggs developed normally.
7	“	35–36	¾ “	Abnormal cleavage, no gastrulation.
8	“	“	1 “	Development stopped at the eight-cell stage.
9	2–4 cell.	33–35	2 “	Abnormal cleavage, no gastrulation.
10	32–64 cell.	31–33	2 “	Normal development.
11	“	“	3 “	Four normal embryos; the rest of the eggs died or became very abnormal.
12	“	“	3½ “	All of the eggs became abnormal, none of them developed into tadpoles.
13	Late seg.	33–35	1½ “	Normal development.
13	“	“	2½ “	Normal development.
13	“	“	3½ “	Eggs killed.
14	“	36–37	½ “	Normal development.
14	“	“	¾ “	A few of the eggs developed normally, most of them died in the gastrula stage.
14	“	“	1 “	Eggs killed.
15	“	40–42	¼ “	Eggs killed.
16	Early gastrula.	33–35	3 “	Normal development.
17	“	35–37	1 “	Development stopped when the blastopore was closing in.
18	“	37–38	¼ “	Normal development.
18	“	“	1 “	Eggs killed.

stops their development before gastrulation begins, sections of the eggs show, in many cases, that the greatest injury has been produced in the yolk portion of the egg which is frequently vacuolated and not segmented although the upper part of the egg has divided into a large number of small cells. Hertwig has noticed the same phenomenon in some of his experiments, and in explanation he states as follows: “Dass Froscheier bei erhöhter Temperatur zunächst partiell geschädigt werden und eventuell absterben, ist offenbar auf die verschiedene Organisation der animalen und vegetativen Hälfte der Dotterkugel zurückzuführen. Die animale Hälfte der Dotterkugel ist reicher an Protoplasma und steht in höherem Masse unter der Herrschaft des Zellkerns. Unter der normalen Wechselwirkung von Protoplasma und Kern können aber Schäden, welche eine Zelle erlitten hat, wie durch verschiedene Experimente festgestellt worden ist, wieder rückgängig gemacht werden. In dieser Beziehung findet sich die vegetative Hälfte der Eikugel unter ungünstigeren Bedingungen. Denn hier ist das Protoplasma nicht nur spärlicher zwischen den Dotterplättchen vertheilt, sondern ist auch am ungethielten Ei mehr dem Einfluss des Zellkerns, der in der animalen Hälfte liegt, entrückt; später, nach Ablauf der ersten Furchungsstadien sind die Theilstücke vielmals grösser als die aus der animalen Eihälfte entstehenden Zellen."

When the injurious effects of the heat are not manifested until the eggs gastrulate, Hertwig (3) finds, in *Rana fusca*, that the abnormalities produced are of two sorts: First, those with a large yolk plug in the posterior region; second, those with deformed heads. In all of my experiments on *Bufo*, the abnormal tadpoles, with but very few exceptions, were of the first sort described by Hertwig. In some cases the development of the eggs stopped when the medullary folds were forming and a large yolk plug was found in the mid-dorsal region; in three cases only was the defect in the anterior part of the embryo. My results are more in accord with Hertwig's experiments on *Rana esculenta* than with those on *Rana fusca*, as in his experiments on the former species he obtained a much smaller number of spina bifida embryos than of those with a large yolk plug at the posterior end of the body.

Hertwig (4) finds that the optimum temperature for the development of *Rana fusca* is 20° C. for the unsegmented egg, and that this optimum rises gradually to 24° C. for eggs in later stages of development. He adds: "Offenbar hängt diese Erscheinung damit zusammen, dass mit der Vermehung der Zellen die Kernsubstanz im Verhältniss zum Protoplasma immer mehr zunimmt und dass so das Protoplasma in höheren Masse ihrem Einfluss unterworfen ist." The optimum temperature for the unsegmented egg of *Bufo lentiginosus* is undoubtedly higher than that for *Rana fusca*, and it is probably somewhere near 28°

C. This optimum in increased 2–3° for eggs in later stages of development.

In another set of experiments on *Rana fusca*, Hertwig (4) finds that the maximum temperature to which the unsegmented eggs can be subjected without suffering any injury is 23–24° C., while this maximum is increased to 30° C. for eggs in the late segmentation stages. The maximum temperature for unsegmented eggs of *Rana esculenta* Hertwig finds to be 33° C. This is also the maximum temperature I have found for unsegmented eggs of the toad, although eggs in the blastula stage can endure a temperature of 38° C. for a very short time.

Morgan has noted that the blastula stages of *Rana palustris* can endure extreme cold much better than can eggs in the 2–4-cell stages, and he also finds that the eggs of *Rana temporaria* which are laid very early in the spring, can survive the tempera- of freezing water for several days. This temperature would very soon kill eggs of *Rana palustris* which are deposited much later than are the eggs of *Rana temporaria.*

While the eggs of all of these species of *Anura* can withstand a wide range of temperature without injury, there appears to be an adaptation to temperature corresponding to the different periods at which the eggs are deposited. *Rana fusca* and *Rana temporaria* lay their eggs very early in the spring when the water is often at the freezing point; and the eggs of these two species can stand cold much better than can the eggs of *Rana palustris* and *Rana esculenta* which are laid considerably later. Although the eggs of *Bufo lentiginosus* are laid but little later than are those of *Rana palustris*, they are usually deposited in shallow pools of water exposed to the direct rays of the sun. They must, therefore, often be subjected to a comparatively high degree of heat during the course of their development.

BRYN MAWR COLLEGE,
BRYN MAWR, PA., April 24, 1903.

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The Relation of the First Plane of Cleavage and the Grey Crescent to the Median Plane of the Embryo of the Frog.

By

T. H. Morgan and **Alice M. Boring.**

With Plate ✓XXII.

Eingegangen am 11. Mai 1903.

The main purpose of our study of the early cleavage of the frog's egg was to find what relation exists between the grey crescent, the first plane of cleavage, and the median plane of the body. We first examined the relation of the position of the first cleavage to the grey crescent in a large number of preserved eggs. Later, in the spring of the year, we determined with living material the relation of the median plane of the embryo to the first plane of cleavage and to the median plane of the grey crescent. The latter results will be given first.

The factors determining the median plane of the embryo in Rana palustris.

Our study of preserved material showed, that while in most cases the first plane of cleavage coincided more or less nearly with the middle of the grey crescent, yet in other eggs it did not, and in a few it even lay at right angles to the median plane of the crescent. This relation made it possible to find out whether the median plane of the embryo follows the first plane of cleavage or the grey crescent.

Each egg was put in a small watch glass having a coat of paraffine on the bottom. Very fine needles were stuck through the jelly and into the paraffine, thus holding the egg without compression in a definite position. An examination showed that the egg held this first position under these conditions. The rusting of the needles did not interfere with the normal development, and was, in fact, of assistance in determining the relation of the plane of cleavage to that of the embryo. In one set, fine glass fibres were used, but proved less satisfactory than the needles. A mark was made on the paraffine indicating the position of the first furrow, and in all but the first set of eggs, a cross was placed opposite the centre of the grey crescent. The eggs used were in the first cleavage stage, or before the second furrow had completed itself, so that there would be no danger of confusing the two. A bunch of eggs was looked over, and those selected in which the first furrow was ninety degrees from the centre of the grey crescent. These eggs were fastened in the paraffine dishes and left until the dorsal lip of the blastopore appeared. Then the needles were carefully removed and the position of the dorsal lip in relation to the first furrow determined. In the first bunch, twenty-eight eggs out of 463 had the first furrow about ninety degrees from the centre of the crescent. When they were examined for the position of the dorsal lip, nine were found either injured, or not yet developed far enough, seven had the blastopore ninety degrees from the first furrow, six in the plane of the first furrow, five forty-five degrees from the first furrow, and one sixty degrees. In this set we were not as careful as later to select only those eggs in which the first furrow was exactly ninety degrees from the centre of the crescent. So that in those eggs in which the dorsal lip appeared ninety degrees from the first furrow, the median plane need not have coincided exactly with the centre of the crescent, and those in which the dorsal lip appeared less than ninety degrees from the first furrow may have more or less nearly coincided with the centre of the crescent. Nevertheless the results show that when the first furrow and the grey crescent did not coincide, there was much discrepancy between the position of the median plane of the embryo and of the first plane of cleavage, and in some cases the median plane of the embryo appeared to coincide with the grey crescent 1).

The number of eggs with the first furrow at right angles to the grey crescent was remarkably similar in the different bunches, being one in every sixteen in the first, one in every fourteen in the

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1) In one in which the centre of the crescent was marked as forty-five degrees from the first furrow, the blastopore appeared forty-five degrees off, thus following the centre of the crescent.

second, and one in twelve in the third. In the second lot, we used the glass instead of needles, and the results were unsatisfactory. In the third lot, we used only those eggs which had the first furrow at right angles to the centre of the crescent, and the crescent side of the egg also was marked. The crescents in this set were much more distinct than in the other sets. Of the thirty-four eggs in this experiment, two had not yet formed the blastopore when examined, three had the dorsal lip in the plane of the first furrow, six ninety degrees away, that is, coincident with the centre of the crescent, three eighty degrees, one seventy-five degrees, one sixty degrees, three forty-five, one thirty, one twenty, and one fifteen degrees away from the first furrow. In two other eggs where the angle between the first furrow and the crescent was only forty-five degrees, the blastopore in one followed the crescent, and in the other neither the crescent nor the first furrow. In every case the blastopore appeared on the crescent side of the egg.

As a check experiment, we fixed twenty-nine eggs of another bunch, in which the middle of the crescent coincided exactly with the plane of the first furrow. In all of these but one, which was accidentally stuck, the blastopore formed in the plane representing both the first furrow and the centre of the crescent. To see whether this bunch was different from the others, we fixed five eggs in which the first furrow was ninety degrees from the crescent; in four eggs the dorsal lip appeared at the centre of the crescent, i. e., ninety degrees from the first furrow, and in one, in the plane of the first furrow.

The Relation of the Planes of Cleavage and the Grey Crescent.

A number of observers, M. SCHULTZE, ROUX, RAUBER, EYCLESHYMER, MORGAN and TSUDA, and others, have studied the variations in the method of cleavage of the frog's egg; but the precise numerical relation between the different forms of cleavage has not been very throughly examined. This is all the more desirable, since the more recent work of SCHULTZE, MOSKOWSKI, KATHARINER, and MORGAN has shown the presence of a new factor in the egg's development, namely, the appearance of the grey crescent on the side of the egg which corresponds to the region where the future anterior part of the embryo will develop. It is true that this same region was sufficiently identified by ROUX, as that part of the egg at which the white reaches its highest point; this condition being due, as he thought, to the oblique position assumed by the egg after fertilization; the egg turning toward that side at which the spermatozoon entered, in consequence of which the yolk-hemisphere of the opposite side is lifted somewhat above the primary equator of the egg. This wedge-shaped region (crescentic in surface view) is the region in which, in many eggs, the grey crescent appears. The change in color that takes place at this time is due to some rearrangement taking place in the interior of the egg. It has been shown by MORGAN that this grey crescent does not appear in eggs that are kept constantly in motion from the time they are taken from the oviducts, through the fertilization and early segmentation periods. Although an embryo forms on these rotated eggs this result need not necessarily mean that when in other cases the crescentic region in question is produced it may not then become the predominant factor that determines either the future of the first cleavage, or the median plane of the body of the embryo, or both. We undertook, in the first place to examine in several bunches of eggs, in which the grey crescent was particularly well developed, the relation of the cleavage planes to the grey crescent, and in the second place, to examine the relative sizes of the four upper cells in the eight-cell stages in their relation to the crescent.

Eggs of Rana palustris in the eight-cell stage were preserved in 3 % formalin. The grey crescent remained evident, although not quite with the same clearness as in the living egg. Out of a hundred and ninety-seven eggs, the crescent could be seen in all the four-cell stages, and in all but twenty-two of the eight-cell stages. In these twenty-two eggs, it is assumed in our calculations that the grey crescent corresponds to the »white crescent«, i. e., that portion of the yolk-hemisphere that lies nearest to the point of meeting of the first and second furrows. This region can also be located from the relation of the pigment to the lower point of meeting of the first two cleavage planes. On one side the pigment comes nearer to this point than on the opposite; vis-à-vis, from this black region, the region of the grey crescent is formed. The two main conditions which were examined were the following:

1) The relation of the smallest of the four black cells at the upper pole in the eight-cell stages to the furrow or furrows passing through the grey crescent.

2) The relation of the highest point of the grey crescent to the first and second cleavage furrows.

Out of a hundred eggs examined in the eight-cell stage, the majority had one black cell smaller than the other three black cells, and this smallest cell was always on the side of the grey crescent. In fifty eggs, one furrow cut through the centre of the grey crescent, thus causing two black cells to border on it, as the crescent in these cases seldom extended more than half way round the egg (Fig. 2). Of these eggs, twenty-nine had the smallest cell to the left of the furrow (Fig. 1) (assuming the egg in a position with the crescent towards the observer), eighteen had the smallest cell to the right of the furrow (Fig. 2), while in three, it was impossible to determine whether the right or left cell was smallest, as in both the third cleavage furrow had come in vertically instead of horizontally (Fig. 3). In the majority of cases the smallest cell lies at one end of the »cross furrow« at the top of the egg, i. e. the smallest cell has a pointed and not a truncated upper end (Fig. 2), but this is by no means always the case, since in eight out of forty-seven eggs, the upper end was truncated as in Fig. 1.

In the other fifty eggs out of the hundred examined, the crescent was not divided in the centre by a furrow. These eggs fell into three classes, those in which the two furrows were equidistant from the middle of the crescent (Fig. 4); those in which one furrow lay slightly to the right of the centre of the crescent (Fig. 5), and those where it lay a little to the left of the centre (Fig. 6). In the first class there were sixteen; in the second, sixteen; and in the third, eighteen. Usually portions of three cells bordered on the grey crescent, one being nearer the centre of the crescent. In a few cases, the crescent did not extend half way around the egg, so that only one cell and part of another bordered on the crescent (Fig. 6). In all but seventeen of these fifty eggs, the black cell lying nearest the centre of the grey crescent was the smallest, or at least not any larger than the others. In fifteen of the exceptions, the third cleavage furrow had come in vertically in that cell, so that it was impossible to say which was smallest (Fig. 5). Only two had this centre cell distinctly larger than the surrounding cells (Fig. 7). It was found here also that the smallest cell was more apt to be pointed than truncated. The majority were pointed, but the difference in number was not so great as when the furrow divided the crescent equally, the proportion this time being fifteen to twenty.

So many cases having been found in which the third cleavage furrow came in vertically, the question arose as to whether this is more apt to occur in one cell than in another; and whether it frequently occurs in more than one cell in the same egg. Out of the hundred eggs, thirty-five were found in which one or more cells had the third furrow vertical. The majority had this in only one cell (Fig. 5), seven had it in two (Fig. 3), four in three (Fig. 8) and one in all four cells (Fig. 9). Those eggs that had divided vertically in three or four cells had lost their circular shape, and become slightly elongated (Figs. 8 and 9), and rather flat on top. As to the cell in which vertical cleavage is most apt to occur, the following results were obtained: in the eggs where the furrow cuts the crescent equally, the proportions for different cells were too close to make it possible to lay more stress on one than on the other; but in those where the crescent was not equally divided, the dark cell near the middle of the crescent was most often divided vertically, namely, in thirteen cases out of thirty-eight. The cell opposite this central cell, and the cell to the right side of the central cell came next in order, there being nine of each. In seven cases the cell on the left side was divided vertically. These numbers are too small, however, to show with any probability that one cell is more liable to this sort of division than another.

In fifty of these eggs in the eight-cell stages, the shape of the grey crescent was examined. It varied greatly in length and width. Sometimes the horns were pointed (Fig. 10), and sometimes cut off abruptly at a furrow (Fig. 11), thus producing anything but a crescent effect. But almost always the crescent reached a little nearer the upper pole in one region than in any other. In the few cases where this was not so, the crescent was bisected by a furrow, and the crescent was at any rate slightly wider at that furrow than elsewhere (Fig. 12). This we shall call the highest point of the crescent. Sometimes this highest point was at the middle of the crescent, thus making the crescent symmetrical in shape (Fig. 11), and sometimes it was to one side of the middle, making an unsymmetrical crescent (Fig. 10). Whether symmetrical or unsymmetrical, however, in all but five eggs, the highest point coincided with a furrow. Those five exceptions were eggs in which the crescent was symmetrical, and not divided in half by a furrow. Of the fifty eggs examined, twenty-four had unsymmetrical crescents. All but two of these had the highest point of the crescent coincident with the furrow nearest the middle of the crescent (Fig. 10). Those two having the highest point coincident with the furrow farthest away from the middle of the crescent, were very unsymmetrical in shape (Fig. 14).

The question then arose whether it is the first or the second furrow which coincides with the highest point of the crescent. To determine this, eggs must be examined in which the second furrow has not quite completed itself, so that it can be distinguished from the first furrow. An examination of ninety-seven eggs in this stage showed that the highest point of the crescent might coincide with either the first or the second furrow, but coincided with the first (Fig. 12) much more frequently than with the second (Fig. 11), the proportion being seventy-nine to thirteen. In these eggs, as in the eight cell stages, there were five cases in which the highest point was not coincident with any furrow (Fig. 13). Again there were more symmetrical than unsymmetrical crescents, fifty-three to forty-four. In the symmetrical, the furrow bisecting the crescent might be either the first (Fig. 12) or the second (Fig. 11). In the unsymmetrical, only a few of the eggs had the highest point coincident with the furrow farthest from the middle of the crescent, they were five in number (the coincident furrow in three being the first, Fig. 15, and in two the second, Fig. 14), and in twenty-five cases, the furrow was slightly to the left of the middle of the crescent (Fig. 16), while in seventeen cases, it was slightly to the right (Fig. 10), thus showing the same majority for the left side that appeared in the eight cell stages.

Comparing these observations with those made by Morgan and Tsuda on Rana temporaria, some differences appear. They make no mention of eggs in which the furrow bisects the crescent, and although the eggs they used did not show the grey crescent, we are assuming it to coincide with the highest region of the yolk hemisphere (»white crescent« of Morgan). They say »the first furrow does not seem to cut the pigment zone bilaterally« and »of the four cells into which the egg is divided, one cell has always the greatest amount of pigment«. In Rana palustris, we found 50% of the eggs had the pigment zone (opposite the grey crescent) cut bilaterally, for, when the crescent is cut bilaterally, so is the pigment zone, and this same 50% had two cells containing more pigment than the other two, but no one cell containing the most.

The three types of cleavage that Morgan and Tsuda mention correspond to the three types described here, in which the furrow does not bisect the crescent, but the percentages differ. If type A be those in which one cleavage furrow is slightly to the right of the centre of the crescent (Fig. 5), type B those in which it is slightly to the left (Fig. 6), and type C where the two furrows are equidistant from the centre (Fig. 4), MORGAN and TSUDA found 64 % of A, 25 % of B, and 11 % of C, while here we found 32 % of A, 36 % of B, and 32 % of C.

Another difference is, that, according to MORGAN and TSUDA, in type A it is always the first furrow that is nearest the centre, and in B it is always the second; while we found that of seventeen eggs of type A, five had the second furrow nearest the centre, and of twenty-seven of type B, twenty-six had the first furrow nearest the centre.

The difference in the two sets of observations may be due to their being made on different species, Rana temporaria and Rana palustris; or the bunches of eggs used in the two cases may have happened to have different variations in cleavage. To see whether the difference in species accounts for the difference in result, some eggs of Rana temporaria also were examined. Fifty-four eggs in the eight-cell stage, and forty before the second furrow was complete, were examined. These eggs had been kept in alcohol for ten years and were somewhat shrivelled, and quite hard to work with; also the grey crescent did not show; so these observations are subject to some error. However, every kind of variation present in the Rana palustris was found here, with but one exception, namely, where the third cleavage plane comes in vertically in all four cells. This being so extreme a variation, and only once occurring among a hundred eggs in Rana palustris, it can not be taken into account as showing any marked difference between the two species. But a decided difference in the percentages of eggs following the different types of cleavage was found, as given in the following table:

	R. palustris	R. temporaria
Furrow through centre of crescent	50 %	24 %
Smallest cell left	58	54
Smallest cell right	36	15
Vertical cleavage in right and left	6	23
Crescent not bisected	50	76
Centre cell larger than others	4	5
Furrow to the right	32	32
Furrow to the left	36	22
Furrow equidistant from centre	32	46

	R. palustris	R. temporaria
Eggs with 3rd cleavage vertical	35 %	57 %
in 1 cell	66	55
in 2 cells	20	42
in 3 cells	11	3
in 4 cells	3	0
in »central« cell	40	45
Symmetrical crescent	52	32
Unsymmetrical crescent	48	68
Highest point of crescent coinc. w. a furrow	90	95
- - - - not coinc. w. a furrow	10	5
- - - - coinc. w. 1st furrow	80	67
- - - - - - 2nd -	15	28
- - - - near centre	95	85
- - - - not near centre	5	15

The most striking differences are in the number of those in which the cleavage furrow bisects the crescent, it being only 24 % in R. temporaria while it is 50 % in R. palustris. This shows a much nearer approach to Morgan and Tsuda's results. In both the smallest cell is more often to the left of the furrow than the right, but in R. temporaria a larger percent have both right and left cells divided vertically, so that it can not be told which cell of the upper four is the smallest. In the eggs where the furrow does not bisect the crescent, the smallest cell is in both species usually the cell at the centre of the crescent, and the percentages of exceptions are almost the same. The percentage of the three types of cleavage, where the furrow does not bisect the crescent, differ in the order in which they come, type B, where the furrow is slightly to the left of the middle of the crescent, being most numerous in R. palustris, while type C, where the furrows are equidistant from the middle as in R. temporaria. In R. temporaria, there are more cases where the third furrow is vertical. Since there are fewer eggs of R. temporaria in which the crescent is bisected, there are fewer with a symmetrical crescent. In both, the highest point of the grey crescent usually coincides with a furrow. In R. temporaria, a few more coincide with the second than in R. palustris, but not nearly so many as with the first. Also a few more eggs have the highest point coincident with the furrow not nearest the middle of the crescent.

These results on R. temporaria more nearly correspond to Morgan and Tsuda's on Rana temporaria. There still remains the same kind of differences, but the numerical value of these differences is less.

In one of the bunches of eggs of Rana palustris that we used in the experiments to determine the relation of the median plane of the embryo to the first cleavage plane and the grey crescent, we noticed some eggs in which the first furrow did not divide the egg into two equal cells. This unequal division occurred in eleven out of one hundred and ten eggs. The grey crescent was partly on both cells in five eggs, and entirely on the larger cell in the remaining six, but never entirely on the smaller.

In this same bunch, the third cleavage came in normally, that is horizontally in all four cells, in only five eggs of forty-four; six had vertical cleavage in one cell, fifteen in two, thirteen in three, and five in four cells. This bunch had been brought to the laboratory before segmentation began and placed in a flat dish. This flattening of the bunch would bring more pressure to bear on the eggs than when the bunch floated in the pond. It is possible that this change in pressure relations may have had something to do with the large percentage of eggs in which the third cleavage came in vertically. It has often been shown that pressure can cause the third cleavage plane to change thus. It may be that all cases of vertical third cleavage are due to pressure changes, but this has not been proved.

Summary.

1) When the first plane of cleavage coincides with the median plane of the grey crescent, the median plane of the embryo coincides with these two.

2) When the first plane of cleavage is at right angles to the median plane of the grey crescent, the median plane of the embryo usually coincides either with the first plane of cleavage or with the median plane of the grey crescent, but sometimes with neither.

3) The dorsal lip of the blastopore appears on the grey crescent side of the egg.

4) In fifty percent of the cases the first plane of cleavage coincides with the median plane of the grey crescent. In about eight and a half per cent of the cases the second plane of cleavage coincides with the median plane of the grey crescent.

In the remaining eggs, the first plane of cleavage lies to one side, or the other, of the middle of the grey crescent, usually near to it.

5) The smallest black cell of the four at the eight cell stage always lies on the crescent side of the egg. When the crescent is bisected by the first cleavage plane, the smallest cell lies in eighteen percent to the right, and in twenty-nine percent to the left. When the first cleavage plane does not bisect the crescent, the smallest cell lies nearest the centre of the crescent.

Bryn Mawr, Penn., April 29, 1903.

Zusammenfassung.

1) Fällt die erste Theilungsebene mit der Medianebene des grauen Feldes zusammen, dann stimmt auch die Medianebene des Embryo mit diesen beiden überein.

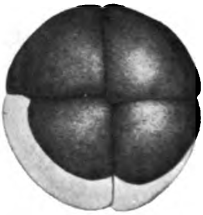
2) Steht die erste Theilungsebene rechtwinkelig zu der Medianebene des grauen Feldes, so fällt die Medianebene des Embryo gewöhnlich mit der einen von beiden zusammen, manchmal jedoch mit keiner von beiden.

3) Die dorsale Blastoporuslippe erscheint an derselben Seite des Eies, wie das graue Feld.

4) In 50% der Fälle fällt die erste Theilungsebene mit der Medianebene des grauen Feldes zusammen. In ungefähr 8½% ist dies mit der zweiten Theilungsebene der Fall.

Bei dem Rest der Eier liegt die erste Theilungsebene auf der einen oder der anderen Seite von der Mitte des grauen Feldes, gewöhnlich nahe derselben.

5) Die kleinste der vier schwarzen Zellen auf dem Achtzellenstadium liegt stets auf der Feldseite des Eies. Wird das Feld durch die erste Theilungsebene des Eies in zwei Theile getheilt, so liegt die kleinste Zelle in 18% der Fälle rechts, in 27% links. Theilt die erste Theilungsebene das graue Feld jedoch nicht, so liegt die kleinste Zelle ganz nahe dem Centrum des Feldes.



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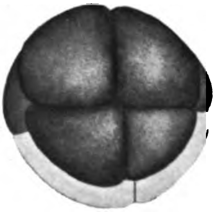

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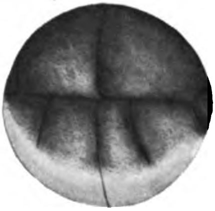

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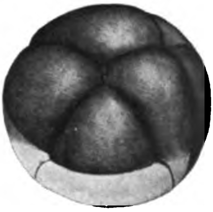

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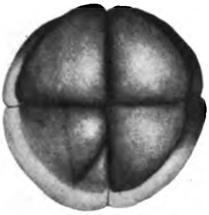

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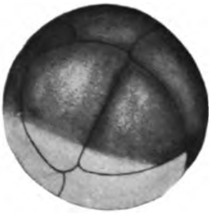

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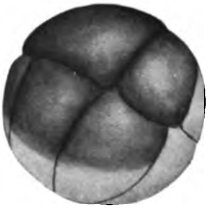

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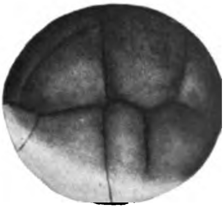

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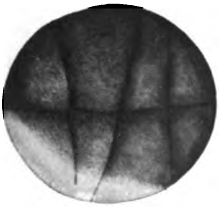

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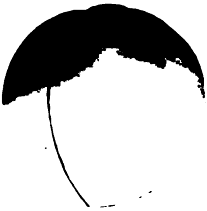

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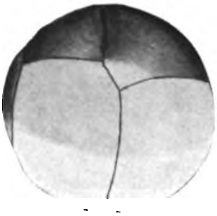

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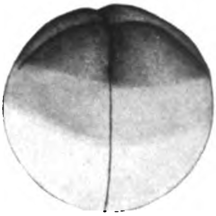

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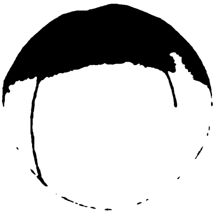

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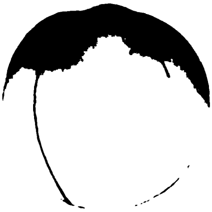

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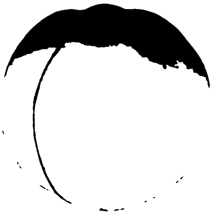

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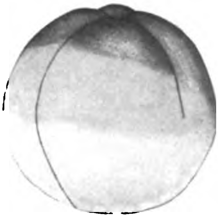

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REGENERATION OF THE LEG OF AMPHIUMA MEANS.

T. H. MORGAN.

My object in studying the regeneration of the limbs of *Amphiuma means* was to discover whether the limbs, which appear to be of so little use to the animal as organs of locomotion, have the power to regenerate as have the limbs of other urodele amphibia.

The first amphiuma that I obtained (in 1900) was a large individual, and after several months had begun to regenerate, but died as the result of an accident before regeneration had gone very far.[1] The next individual that I was able to procure was also large, but escaped before regeneration had gone any farther than in the last case. Two smaller individuals have been kept for more than a year (from March 21, 1901, to May 3, 1902). The following account applies to them. Each had a fore-leg and hind-leg of opposite sides cut off through the upper portion of the leg. In the course of several weeks a knob of new tissue appeared which continued to elongate for several months, when further growth seemed to have ceased. To make certain of this, the animals were kept for six months longer, but no further change occurred. The new part was shorter than the part removed, and appeared to be a single rod, tapering at the end, without any external signs of toes.

The normal fore- and hind-foot of the amphiumas that I used had each three toes. Cope[2] gives a figure of the skeleton of amphiuma showing a cartilaginous carpus of four or five pieces, and three ossified metacarpals with ossified phalanges. In the hind-foot there are three cartilaginous tarsalia, three ossified metatarsals and three phalanges.

After the legs had regenerated they were cut off, imbedded in paraffine, and cut into sections. These showed in three of the four cases that the two bones of the middle part of the limb have developed. The condition of the carpus and tarsus appears to be different in each of the four cases, Figs. 1–4. The rough reconstructions shown in these figures were made from sections. The figures are not very accurate, but serve to show the number of bones and their relation to each other. The relative sizes of the bones is less exact. It will be seen from the figures that the regeneration has lead neither to the formation of a uniserial row of skeletal elements, nor is it clear in all cases whether more than a single toe is represented. It seems probable that the terminal middle phalanx represents a toe, but whether any of the other cartilages represent other suppressed toes can not be stated.

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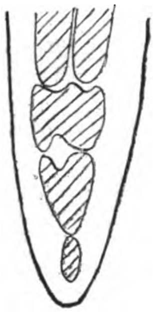
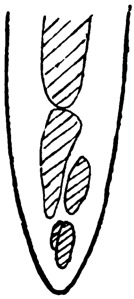
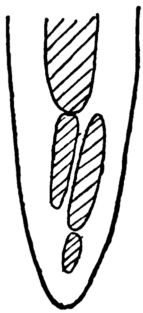
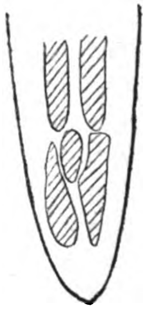
[1] This is the case referred to in Towle's paper. BIOLOGICAL BULLETIN, II., 1901.

[2] Cope, "The Batrachia of North America," Bull. U. S. Nat. Mus. No. 34.









In these four cases the legs had been cut off through the humerus, or the femur. It occurred to me that if the limb were cut off through the fore-arm or the fore-leg the result might possibly be different, since two bones are present at the cut surface. Therefore on May 3, 1902, when the two regenerated legs were removed for study, the remaining two legs were cut off through the fore-leg and fore-arm.

The two amphiuma were kept alive for nearly another year; until March 30, 1903. They were occasionally fed on earthworms. The limbs that had been cut off through the fore-arm and fore-leg regenerated, but again produced only a single pointed, or in one case a somewhat flattened, new part. Serial sections show that, besides completing the ends of the two bones at the exposed surface, there have been produced a number of more distal cartilages. The arrangement of these pieces is irregular, and different in each case, as also occurred when the leg was cut off through the upper portion. In other words, no better regeneration took place here than in the former instances.

It is also of interest to notice that the other two legs that had been cut off (close to the body) for examination had not regenerated. The skin grew over the cut surface, and in several cases the muscles of the body wall seemed to have grown over the short piece of the humerus or femur that had been left. At most, a short protrusion indicated the position of the limb.

How shall we interpret this result. Those who hold that the power to regenerate a part is commensurate with the value of the part to the animal, if it is a part liable to injury, will welcome this experiment as in harmony with their interpretation. On the other hand, as I have tried to show elsewhere, the evidence is so strong against this point of view that I think we shall not go wrong if in this case we deny that the result has any such meaning.

In fact, in other adult amphibia, in the frogs for instance, in which the limbs are of some importance to the animal they cannot be regenerated, although in the tadpole stage in which the limbs are of no importance, and, in the case of the fore-limb at least, not liable to injury, the power of regeneration is present. Moreover even in the urodeles the power of regeneration is unequally developed in forms that use their legs for purposes of locomotion. It is said that *Triton marmoratus* shows only a slight power to regenerate its legs. In other cases, as I have observed in *Necturus*, the time required to regenerate a leg is so long that were the presence of the leg essential to the existence of the individual it would succumb before the regeneration could take place.

These considerations make it clear, in my opinion, that the lack of complete power to regenerate in amphiuma can not be interpreted as having any connection with the unimportance of the legs to the animal. It should not be overlooked that it is not that the leg does not regenerate at all; in fact it regenerates quite well, but that the new part is different from the old. It is at least conceivable that some simple physical or physiological factor may interfere with the formation of the complete toes, such, for instance, as the thickness of the skin in relation to the size of the limb.

If it could be shown that the leg of amphiuma is a degenerate structure it might appear that there is some connection between the degeneracy of the part and its lack of power to regenerate, but it is far from being established that any such general relation really exists. In fact, in the male hermit crab I found that the very small and *apparently* rudimentary abdominal appendages have the power to regenerate. It would be interesting, nevertheless, to examine this point further in cases where the degeneration and uselessness of an organ are more certainly established, as in the case, for example, of the appendix of man, which does not appear to have the power to regenerate after removal.

Woods Holl, Mass., June 22, 1903.

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Further Studies on the Ciliate Infusoria, Licnophora and Boveria.

By

Nettie Maria Stevens.

(Hierzu Tafel I—VI.)

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Introduction.

In an earlier paper[1], I gave the results of my work on the two new species of Infusoria, Licnophora macfarlandi and Boveria subcylindrica, both found in the respiratory organs of Holothuria californica STIMP., at Pacific Grove, California. Since that time I have had an opportunity to study other species and varieties belonging to these genera, at the Marine Biological Laboratory, Woods Hole, Massachusetts, and at the Naples Zoological Station. The present paper will be devoted: (1) to a comparative study of the species of each genus, and of their relation to other ciliates; (2) to observations on the conjugation, regeneration, and response to electrical stimulus in Licnophora; and (3) to a description of the division of the micronucleus and of the formation of a new peristome in Boveria.

Licnophora.

Historical summary. — As was stated in the historical summary of my former paper ('01), Licnophora was first described, but not named, by CLAUS ('62), as a parasite on Cladonema. The genus Licnophora was created by CLAPARÈDE ('67) for two new species, Licnophora auerbachii COHN and Licnophora cohnii CLAP. GRUBER ('84) gave the name Licnophora asterisci to a form which he found on Asteriscus, and described as very near to Licnophora auerbachii. FABRE-DOMERGUE ('88) found Licnophora on Syllis and Ophiothrix, and expressed the opinion that all the species described should be united under the name Licnophora auerbachii; but WALLENGREN ('94), who worked on Licnophora from Doris muricata, the host on which it was discovered by Auerbach, thinks that Fabre had a still different species. Two fresh water forms were described by Maskell ('86) and Garbini ('98), but these forms evidently do not belong to the genus Licnophora. Licnophora macfarlandi was found in the respiratory organs of Holothuria californica Stimp., by Prof. F. M. Mc Farland in 1893. During the summer of 1901, while working at the Marine Biological Laboratory, Woods Hole, I learned from Dr. Conklin[1]) that what proved to be another new species of Licnophora occurred on the eggs of Crepidula plana. This species had also seen by Dr. Calkins, and is described by him in a recent bulletin of the U. S. Fish Commission, as a variety of Licnophora macfarlandi ('01[2]).

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[1] Studies on Ciliate Infusoria. Proc. of the Cal. Acad. of Sciences. Ser. III. Zool. III 1901.

The species of Licnophora thus far recognized with their hosts are as follows: —

L. auerbachii Cohn	on Doris muricata (Auerbach, Cohn, Wallengren).
	„ Thysanozoon tubercula (Clap.).
L. cohnii Clap.	„ Psyrmobranchus protensus (Clap.).
L. asteriscus Gruber	„ Asteriscus (Gruber).
L. (auerbachii) Fabre	„ Syllis and Ophiothrix (Fabre).
L. macfarlandi Stevens	„ Holothuria californica (Mc Farland, Stevens).
L. conklini Stevens	„ Crepidula plana (Conklin, Calkins, Stevens).

Licnophora macfarlandi. — A brief summary of the description of this species given in my former paper will serve to recall the principal characteristics of the genus together with the specific peculiarities of the species. This ciliate has an elongated body which is divided into three distinct regions, — the attachment disc, the neck or stalk, and the oral disc (Fig. 1, a, b, c). The attachment disc is nearly circular in outline, and consists of a shallow circular cup surrounded by four ciliary membranes (Fig. 1, m^1—m^4), whose cilia, though usually united, may be separated at any point without destroying the coördination of their movements. Outside of the membranes is a collar or velum, consisting of a longer dorsal, and a shorter and wider ventral portion (Fig. 1, v). The neck is flattened dorso-ventrally, and has on the ventral side a longitudinal furrow leading to the mouth. On the right-hand side there is a vibrating lateral membrane extending from the point in the border of the attachment disc where the two parts of the velum meet, to a point just within the oral band near its origin over the pharynx (Fig. 1, e). Being very flexible, the neck varies greatly in length and thickness at different times, and in a contracted state shows conspicuous wrinkles and furrows on both dorsal and ventral sides (Fig. 1; and '01, Pl. I, Figs. 2 and 3).

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[1]) The name Licnophora conklini has been given to this species.

The oral disc is oval in outline, concavo-convex, and has, on the slightly concave ventral surface. a left-turning peristomal circlet, consisting of very long fine cilia disposed in about one hundred and twenty-five short rows at right angles to the direction of the spiral (Figs. 1 and 3). This ciliary band begins at a point near the border between the neck and oral disc on the right-hand side of the animal, extends around the anterior end and left side of the disc, turns to the right, and passes with a twist of 180° into the mouth and pharynx, which together form a pear-shaped cavity with a large external opening, variable in form and size (Fig. 1, o). The oral band has a very complicated structure, as is shown by sections and macerations. Each cross row of cilia originates in a basal band which stains like the basal bodies of single cilia (v. LENHOSSÉK '98; PETER '99). The ends of these basal bands are connected by delicate fibres with a long stout fibre which extends from the base of the attachment cup, in which it has root-like branches, to the end of the oral spire in the pharynx (Fig. 3; and '01, Pl. II, Fig. 17). The cilia of each row are usually twisted together when in action so as to resemble membranellae, and are figured as such by WALLENGREN ('94) and CALKINS ('01^2). When, however, the organism is viewed under a high-power immersion lens, it is evident that the cilia are distinct. The basal structures indicate that we may have here an intermediate stage in the formation of membranellae from distinct cilia, giving such striated structures as form the peristomal circlet of Stentor and of many of the Hypotrichae. It is possible that united cilia may be found in some species of Licnophora, but such is not the case in the forms that I have examined. Another smaller fibre extends from the point in the border of the attachment disc where the lateral vibrating membrane begins, to the oral end of the peristomal band (Fig. 3, f^2). These fibres, though slightly contractile, seem to function rather as supporting structures than as myonemes.

A single micronucleus occupies a somewhat isolated position at the base of the attachment cup, to the left of the larger neck fibre (Figs. 3 and 16; '01, Fig. 4).

The macronuclear chain consists of from twenty-five to thirty-five segments in adult specimens, and may be continuous or broken into several sections. The segments are concentrated into one or more spherical masses before division. When writing my previous paper, I thought that the segments were completely separated in the adult; but, after working on other species where connection between the segments is clearly evident, and examining some preparations of Licnophora macfarlandi that had not been carefully studied, I have concluded that in this species the yare also connected, either in a single branched chain or in as many groups as there are separate nuclear masses in division.¹) The connection is, however, very difficult to detect, and, it appears, may be easily broken (Fig. 16; and '01, Fig. 31).

I also thought that, as Wallengren ('94) had stated, the macronuclei unite in pairs before concentration and division; but this I find from study of the other species is not the case: instead, there is a division of the chromatin in each segment before the segments unite (Figs. 6 and 20; and '01, Figs. 15 and 19).

Fission in Licnophora is longitudinal, a new oral spiral being formed on the left side. The division line runs between the old and new peristomes, and through the attachment disc ('01, Pl. III, Figs. 18—28). The new peristome first appears as an oval ciliary field, from which is formed a right-turning spiral that later turns to the left ('01, Figs. 19—27). The micronucleus migrates to the anterior end of the oral disc, takes a position near and usually anterior to the concentrated macronucleus (Fig. 4). There it divides, and the two resulting micronuclei remain near the ends of the elongated macronucleus during its division, then return to their normal position near the base of the attachment cup of each individual. Division of the cell proceeds from the anterior end through the oral disc, neck and attachment disc. The macronuclear band or bands, resulting from division, take the adult position in each individual and separate into segments ('01, Figs. 18—31).

A contractile vacuole is not present, and defecation has not been observed.

This ciliate is usually attached to its host. The movements of its cilia, ciliary membranes and lateral vibratile membrane are such as to send a food current to the mouth by way of the ventral furrow in the neck, and also from the opposite direction, when the

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¹) In one case eleven such masses were counted, and from two to five are more common than one.

oral cilia are clapped down on the ventral surface, as they are at intervals in feeding.

The animal is usually rotating from left to right, and turning its oral disc this way and that by means of muscular movements of the neck. When disturbed, it swims rapidly away with the oral disc at right angles to the attachment disc, darting hither and thither; or it whirls round and round with the two discs in nearly the same plane. It also moves short distances on the surface to which it is attached, and occasionally is seen running about on the host by means of its oral cilia and ciliary membranes with Trichodina-like agility; a method of locomotion recently observed in Stentor by Jennings ('02).

The food of this species is usually diatoms, but may also be desquamated cells from the epithelial lining of the respiratory organs of its host. It has even been known to take in and digest small specimens of Boveria in cases where there was an immense number of infusoria of both kinds in the small terminal branches of the respiratory organs, and "a struggle for existence" was literally going on.

This species differs from those previously described (1) in being an endoparasite, (2) in having four ciliary membranes around the attachment cup, (3) in having a stout axial fibre connecting the attachment disc, to which it sends branches, with the oral ciliary band with whose rows of cilia its branches are connected, and (4) in having a lateral vibratile membrane. The micronucleus had not been previously described in any species of Licnophora.

3. *Licnophora conklini.* — This form, which occurs on Crepidula plana and on its egg-capsules, appeared at first sight to be identical with the Monterey species, but more careful observation showed that the vibratile neck membrane, so conspicuous in Licnophora macfarlandi, was not present, though the right side of the neck showed something of the same movements, being raised and lowered at intervals to guide the food particles down the neck furrow into the mouth. This Licnophora is somewhat smaller than the Pacific coast species, and there is a much greater difference in size between young specimens and those in the first stages of division (Figs. 6, 7, 11, 12; and '01 Figs. 28 and 19—21).

The movements of this species, when attached and when swimming, were the same as in Licnophora macfarlandi; but individuals were very often seen running about over the egg-capsules, a kind of locomotion rarely observed in the other species. As in the case of

Licnophora macfarlandi, this species differs from that described by Wallingren ('94) in the form of the attachment disc, the number of ciliary membranes, and the structure of the peristomal spire. Neither of these American forms show the "haftring", membranellae, or "peristomalrinne" of Wallengren's description. If the lateral vibrating membrane *e* is omitted, Fig. 1 is a correct representation of Licnophora conklini.

On fixing specimens of this species and staining with Delafield's haematoxylin, alum-carmine or picro-carmine, it was found that there is a striking difference between the macronucleus in this case and that of the other forms described. Instead of being broken up into many segments, forming a long chain extending through the neck and around the two discs, the macronucleus here is divided into four widely separated parts. A band extends from one half to two thirds of the distance around the attachment disc on the dorsal side; there are two segments posterior to the mouth; and a group of usually two segments, not widely separated as in the other cases, lies near the anterior extremity of the oral disc on the right side (Fig. 5). The anterior group is occasionally represented by a single segment and sometimes by three; in rare cases all four parts are segmented (Fig. 22). The segments in this species are all connected as shown in Fig. 5, and only one macronuclear mass was observed in division.

The external phenomena of division are essentially the same as in Licnophora macfarlandi. This species increases in size rather more in early stages (Figs. 5—8), and there appears to be less growth and a greater change in form, involving more shifting of the cytoplasm, in later stages (Figs. 8—10). The young Licnophorae are in some cases very short and broad and gradually lengthen as the macronucleus lengthens and segments (Figs. 11—15), while in other cases lengthening of the body and segmentation of the nucleus occur before the two individuals separate. In this species the two attachment discs always divide before the oral discs.

Either just before or soon after the first cilia of the new peristome appear, there occurs a peculiar nuclear change, referred to above and noted in all the forms studied (Figs. 6 and 20). All the chromatin of each segment is separated into two parts, not necessarily equal. In the band which partly surrounds the attachment cup, there may be three, four or even more such divisions. Between each of the two masses of chromatin thus separated, there is a space where no stainable material is present, and in each mass there is a nucleolus, while ordinarily only one nucleolus is present in each segment (Fig. 20). The nucleoli are not seen in specimens stained with haematoxylin or alum-carmine, but appear in picro-carmine, osmic acid or potassium bichromate preparations. What the significance of this chromatin separation in the earliest stages of division may be, it is difficult even to guess, for all the segments later unite into a spherical mass which shows no trace of this introductory division (Figs. 7 and 8); nor is there any indication in later stages that individuality of the divided segments may persist during the process of gross division of the concentrated macronucleus, and the individual half segments become the nuclear segments of the two young Licnophorae.

As in Licnophora macfarlandi, the micronucleus migrates to the anterior end of the oral disc, and divides mitotically; the two resulting micronuclei remain near the ends of the elongated macronucleus during its division, and then move posteriorly to their usual position at the base of the attachment cups (Figs. 8—12).

A careful search for Licnophora was made on various mollusks, holothurians and worms at Woods Hole, but these ciliates were found only on Crepidula and its egg-capsules.

4. *Licnophora auerbachii.* — Out of the large number of holothurians, echinoderms, mollusks and worms examined at Naples, Licnophorae were found only on Asterina gibbosa, Ophiothrix fragilis, Thysanozoon tubercula, Capsa fragilis and Tellina exigua. The Licnophorae from all of these hosts resemble closely Licnophora auerbachii as described and figured by Wallengren ('94), so far as general form, number of segments in the macronuclear chain, and division phenomena are concerned; they also show many of the distinguishing characteristics of Licnophora macfarlandi and of the Woods Hole species. These characteristics are four ciliary membranes around the attachment cup, a stout fibre connecting the attachment cup with the oral ciliary band, a ventral furrow leading to the mouth, and the peristomal band composed of short rows of long fine cilia whose basal bands are connected by fibres with the common fibre running through the neck to the attachment cup (Fig. 2). All of these structures I was able to recognize in preparations of Licnophora anerbachii which Wallengren kindly sent to me while I was working on Licnophora at Stanford University in 1900. They must, therefore, now be regarded, without much doubt, as generic rather than specific characteristics.

The Naples Licnophorae differ considerably in size on the various hosts, — from 79 μ in length on Thysanozoon to 116 μ on Capsa, the largest specimens, not in division stages, being measured alive when attached to the slide with the two discs in the same plane, as in Fig. 1 and 2. The smallest on Thysanozoon and Asterina were less than half as long as Licnophora macfarlandi, and the largest on Tellina and Capsa slightly smaller than Licnophora conklini. Variations in size may be largely due to differences in habitat, for all of the larger forms are found in protected situations, — in the respiratory organs of Holothuria, or within the shells of Capsa, Tellina and Crepidula, while the smaller forms are on the surface of Asterina, Ophiothrix and Thysanozoon.

The most striking external differences are: (1) the presence or absence of the lateral vibrating membrane characteristic of Licnophora macfarlandi, and (2) the variations in the form of the attachment disc.

As was stated for Licnophora conklini, there is a movement of the thinner right-hand side of the neck which corresponds in some degree to the vibration of the thin extended membrane of the Monterey species.

The attachment disc of the Licnophorae on Asterina and Ophiothrix, when fastened to the slide or cover-glass, always has the form figured by Wallengren ('94) for Licnophora auerbachii (Fig. 2). The diameter is greater in a transverse than in a longitudinal direction, the ratio being 8 : $6^1/_2$. The disc has an irregular outline on the side toward the mouth, and on the right side there is a definite notch where the two parts of the velum and the right side of the neck meet (Fig. 2, d). This notch is less marked in Licnophora macfarlandi (Fig. 1, d). A few of the specimens on Tellina have a disc of the same form, while others on Tellina and all on Capsa and Thysanozoon have a nearly circular disc. The form and structure of the attachment cup, the ciliary membranes and the velum, as well as the relation of the disc to the two parts of the velum and to the neck at the point *d* are the same in all (Figs. 1, 2, 24—26). In the specimens with a round disc, the neck is somewhat narrower, and usually longer and thinner, while in the broad forms the line of attachment of the neck to the disc is much longer (Figs. 24—26). This difference in form and attachment of the neck may account in part at least for the different appearance of the discs of different specimens when attached to the slide, for the disc always appears circular in preserved specimens not attached to a surface. In Licnophora from Thysanozoon the cup is broader in proportion to the diameter of the disc than in any of the others, the ratio being 6 : 8, while in Licnophora macfarlandi it is 6.4 : 11.4, and in Licnophora conklini 5 : 10. This broader cup may be an adaptation to conditions of life on its slimy host.

With the exception of a few specimens from Tellina, which may have been preparing for division, the forms with circular discs all belong to soft-bodied hosts, while those with broader necks and discs are found on the hard spines of Asterina and Ophiothrix. This and the fact that the broader discs differ considerably in form in specimens from the same host, lead me to think that this is not a species characteristic, but an adaptation to the surface to which the organisms attach themselves. It would be an interesting experiment to transfer specimens from Asterina or Ophiotrix to Thysanozoon or to some other soft-bodied host, to see whether adaptive changes in the attachment disc would occur quickly, in which case the variation in form would not be a specific character.

The circular attachment disc, somewhat more slender neck, and shorter oral disc of the Licnophorae on Thysanozoon tubercula might identify this form with that described by CLAPARÉDE ('67) as Licnophora cohnii. This is probably not the case, however, as he called the form which he found on this species of Thysanozoon at Naples Licnophora auerbachii, and figured Licnophora cohnii with a very long slender pedicle and a circular oral disc.

In the Licnophorae from Ophiothrix, the inner ciliary membrane seems to be permanently united and thickened for use as a grasping organ; and this modification is noticeable, but not to the same extent, in those from Asterina.

In all of the Naples forms the shorter ventral portion of the velum is slightly less transparent and therefore more easily distinguished than in Licnophora macfarlandi. It vibrates much more rapidly than the other part of the velum, or the ciliary membranes, and for that reason is difficult to see, lying as it does between the ciliary membranes and the neck.

The oral disc in the Licnophorae on Tellina and Capsa most closely resembles that of the Monterey species; while in those found on the other hosts the disc as well as the neck is somewhat shorter in proportion to the width (Figs. 1 and 2).

Division stages in the Naples forms do not differ materially from those described for Licnophora macfarlandi and Licnophora conklini. The changes in form and size are more like those observed in the former. The method of formation of the new peristome is precisely the same, and the division stages of macronucleus and micronucleus are the same as in Licnophora conklini, with the exception that in rare cases the nuclear chain concentrates into two or three masses for division instead of one, indicating breaks in the chain as in Licnophora macfarlandi. Separation of the two attachment discs before that of the oral discs occurs here as in Licnophora conklini.

The one striking difference on which classification may be based is that found in the macronuclear chain. In the Woods Hole species (Fig. 17) we have what appears to be a more primitive form with four distinct and widely separated divisions of the macronucleus distributed to different parts of the body. In the European forms (Fig. 18) the number of distinct segments varies from ten to twenty-five. These segments are, however, separated into four groups corresponding to the four distinct divisions in Licnophora conklini, and the four sections are sometimes seen in resegmentation after division, but more commonly the two middle sections are united as in Fig. 21. Figure 18, a specimen from Asterina, shows a typical number and arrangement of segments, — (*a*) three corresponding to the band in the attachment disc of Licnophora conklini, (*b*) two, and (*c*) two segments corresponding to the right and left middle sections, and (*d*) six to the peristomal section, which is usually divided in Licnophora conklinii. The number of segments in group *a* may vary in specimens from the same and from different hosts, from three to six, that in group *b* from two to three, in group *c* from one to six, and in group *d* from four to eleven. The largest number of segments observed was twenty-three in one large specimen from Tellina, but another equally large one had only eleven, showing that no necessary relation exists between size of the organism and number of segments. In WALLENGREN's preparations of Licnophora auerbachii from Doris muricata, the variation in number of segments is indicated by the following counts: 13, 16, 17, 20, 21, 22, 25. The variations were from four to seven in the attachment disc and from seven to twelve in the peristomal group. Fourteen and fifteen were the most common numbers in all of the Naples forms. The larger numbers were always seen in large specimens, but such specimens often contained only from eleven to fifteen.

Thus it appears that, if we disregard the differences in size and in form of the attachment disc, all of the Licnophorae studied at Naples may be classed with the one described by WALLENGREN ('94), under one species, Licnophora auerbachii.

Classification. — Though the number of segments in the macronucleus of Licnophora macfarlandi is considerably greater, the same grouping as in the other species can be distinguished, but the groups are not so clearly separated (Fig. 16). The neck is longer in this species than in either of the others, and the middle group of segments is extended toward the attachment disc (Fig. 16, b). The arrangement shown in Fig. 16 is typical, but considerable variation in the number of segments of each group occurs, and the groups are often more closely connected.

The considerably greater size of Licnophora macfarlandi, the larger number of macronuclear segments, and the highly developed vibratile neck membrane lead me to consider it a separate species, until further search can be made on the Pacific Coast, for different species or for the same species on different hosts. A few specimens were found by Dr. HAROLD HEATH at Pacific Grove in 1901 on Cymbulopsis, but they were not observed alive, nor were they compared with the Licnophora from Holothuria. Licnophora may therefore occur on various hosts in Monterey Bay, and if so, it will be interesting to ascertain whether the same or similar variations appear as on the different hosts at Naples.

Until the group is still further studied, I shall refer the forms that I have investigated to the three species, L. conklini, L. auerbachii and L. macfarlandi, and give the following tentative description of genus and species: —

Licnophora. — Length 80—180 μ. Colorless or slightly yellowish. Body flexible [and contractile, flattened dorso-ventrally and consisting of three distinct regions, — attachment disc, neck, and oral disc. Attachment disc circular or irregularly oval in outline. Attachment cup nearly hemispherical, and encircled by four concentric ciliary membranes, and by a velum consisting of a longer dorsal and a shorter ventral portion overlapping on the left side and meeting on the right side at a notch in the attachment disc. Neck flattened dorso-ventrally and varying greatly in width and length. A conspicuous furrow on the ventral side extending from the attachment disc to the mouth. Oral disc oval or nearly circular, concavo-convex, with the sinistral peristomal spire on the concave ventral side. Peristomal band composed of short rows of long fine cilia rooted in basal bands connected with a thick, somewhat contractile fibre which extends to the attachment cup, in whose walls its root-like branches ramify. Micronucleus at the base of the attachment cup to the left of the axial fibre. Macronuclear chain consisting of from four to thirty-five segments, separated into four groups distributed to the attachment disc, neck region and oral disc. Division longitudinal, a new peristome being formed on the left side as a right-turning spiral which later changes to a left-turning spiral. The attachment disc elongates transversely and divides into two equal discs. Conjugation of equal gametes, not permanent. Marine forms. Ectoparasites on various echinoderms, worms, mollusks and medusae; endoparasites in the respiratory organs of Holothuria californica.

L. conklini. — Medium size, 100—135 μ. Attachment disc circular or nearly so. Macronucleus having the four parts usually undivided, except in the peristomal section which, as a rule, consists of two segments. Found on Crepidula plana at Woods Hole, Mass.

L. auerbachii. — Small to medium size, 80—120 μ. Attachment disc either circular or irregularly oval, the ventral side being notched and less curved than the dorsal side. Neck short and broad. Macronuclear chain of from ten to twenty-five segments, separated into four distinct groups containing a variable number of segments; number of segments more commonly fourteen or fifteen. Found on Asterina gibbosa, Ophiothrix fragilis, Thysanozoon tubercula, Tellina exigua, Capsa fragilis and Doris muricata (WALLENGREN).

L. macfarlandi. — Large, 140—180 μ. Attachment disc circular or nearly so. Macronuclear chain showing the same grouping as in the other species, but divided into more segments, twenty-five to thirty-five. A delicate vibratile membrane on the right side of the neck. Found in the respiratory tree of Holothuria californica in Monterey Bay, California.

Relationship of Licnophora to other Ciliates. — CLAPARÈDE ('67), who created the genus Licnophora, regarded these ciliates as true Hypotrichae furnished with an attachment disc; and Trichodina as a form derived from temporarily free-swimming Vorticellae. This classification was based on the fact that Licnophora, like the Hypotrichae, has a left-turning peristomal spire, while that of Trichodina and Vorticella is apparently a right spiral.

BÜTSCHLI ('89) regards Licnophora as a transitional form between hypotrichous and peritrichous infusoria. He derives the Licnophoridae from the Hypotrichae which they resemble in having an arched dorsal surface, and cilia only on the ventral surface. The peristomal spire is a left-turning one as in the Hypotrichae. The other peritrichous forms with left spirals he derives from the Licnophora-type by loss of the posterior circle of cilia and elevation of the peristome to a terminal position. The Urceolaria-type, Trichodina-like forms, he regards as formed from the Licnophora-type by extension of the adoral spire around the whole ventral surface dorsal to the attachment disc. The Vorticellidae are then derived from the Trichodina-type by loss of the attaching circle of cilia and extension of the ventral surface giving a conical form, with the adoral spire around the base of the cone. Looked at from the ventral surface the spiral is still a left-turning one, but from the peristome, which according to this theory is morphologically the dorsal side, the spiral turns to the right. The attaching part is drawn out to form a contractile stalk, but the Trichodina-type appears in the free-swimming modification which acquires a ventral circle of cilia. Division in Vorticella is transverse, two new peristomes being formed from the old one by division and partial regeneration; and BÜTSCHLI predicted that division in Licnophora, which had not then been observed, would also be transverse. The discovery that Licnophora divides longitudinally and that the peristome forms as a right spiral which changes to a left spiral, led WALLENGREN ('94) to reject BÜTSCHLI's theory, and express the opinion that Licnophora must be regarded as a highly differentiated form of peritrichous infusoria, and its relations to other Peritrichae remain for the present an open question.

The recent work of WALLENGREN ('01) on Oxytricha indicates that such changes as occur in the formation of the peristome in Licnophora may have a phylogenetic significance. He finds that the reconstructions taking place in division follow the lines of development of the more complex from the simpler Hypotrichae. In the five different species which he studied, the cirrhi were in every case absorbed before division, and six rows of cilia appeared on the ventral surface, as in the more primitive forms; these six rows of cilia were then transformed into the adult cirrhi.

JOHNSON ('93) also calls attention to the probable phylogenetic significance of the change in the new peristomal band in Stentor from a lateral to a terminal position. In the division of the macronucleus of Stentor, JOHNSON observed no structural changes and concluded that, as division may occur during concentration, at the period of greatest condensation or during re-nodulation, the object of concentration must be merely to secure a larger number of nodes for the daughter animals. He states in this connection, however, that division at the period of greatest condensation is probably the primitive method, a reminiscence of a time when the nucleus was always spherical.

In Licnophora division and rearrangement of the macronuclear segments in the two new individuals could hardly be effected without condensation of the nuclear chain; but the varying number of segments in the different species from four to thirty-five, the arrangement of the segments in four groups in all the species, together with the method of condensation, division and resegmentation, suggest that the primitive Licnophora-type, or the form from which it was derived, had one spherical macronucleus, and that in the course of the development of the present Licnophora-form, the nucleus first became an elongated band, like that of Trichodina and Urceolaria; the band then segmented into four parts, and further segmentation took place later in varying degrees in the different species. Figures 11—15 may illustrate ontogenetically some of the phylogenetic changes in the Licnophora nucleus, while later changes appear in the adult nuclear conditions of the different species (Figs. 17, 18, 16).

The multiple nuclear masses in division, observed in Licnophora macfarlandi and rarely in Licnophora auerbachii, must be attributed to breaks between the segments, due probably to contortions of the organism, and to greater extensibility of the body cytoplasm than of the nuclear membrane. Such breaks would of course be handed down to all descendants of an individual in which they occur, until a new macronucleus is formed after conjugation.

If the changes in the new peristome have such phylogenetic significance as WALLENGREN suggests for Oxytricha and JOHNSON for Stentor, then the three successive stages, — (1) an oval field covered with short cilia of equal length, (2) development of a right-turning spiral by growth and definite arrangement of the outer cilia of the field, while the cilia in the center of the field degenerate ('01, Figs. 11, 16, 19, 20), and (3) change of the right spiral to a left spiral, — would indicate that the present type of Licnophora has been derived from a holotrichous form covered with short cilia of equal length, that the first differentiation of cilia in the oral region took the form of a right spiral, and that the change from a right to a left spiral was probably coincident with a gradual change in the form of the organism, which is repeated in the development of each new individual, but is somewhat obscured by the retention of the old left-turning spiral in the parent organism.

As to the phylogenetic development of the attachment apparatus, nothing is indicated in the phenomena of fission, since the disc, membranes, velum and cup are equally divided between the two individuals; and no regeneration occurs when the attachment disc is removed.

Conjugation in Licnophora auerbachii. — During four summers, three at Pacific Grove and one at Woods Hole, I had watched in vain for conjugation in Licnophora macfarlandi and Licnophora conklini. While examining Licnophora auerbachii on Thysanozoon tubercula at Naples, I discovered one pair in conjugation, but was unable to fix and stain them satisfactorily on account of the slime in which they were embedded. The following day I found a second pair in material taken from Asterina gibbosa, and the next day several pairs were obtained from the same host, an individual that had been in the laboratory six weeks, and on which the infusoria were very abundant. A few days later two pairs were found on Ophiothrix fragilis. There seemed to be no difference in the mode of union of the Licnophorae on the different hosts, and those from Asterina were much more convenient to work with, as the conjugating pairs very quickly attached themselves to the slide and remained in one place until they separated. The slides could therefore be placed in a moist chamber, the pairs observed at intervals, and fixed as desired. In all cases, however, the union was effected before the material was removed from the host, so that it was impossible to tell from the living material what stages one might have, or how long the period of union lasts. One pair which was observed at intervals for seventeen hours, was just separating at the end of that time, but how long they had been united before this period of seventeen hours was of course not known.

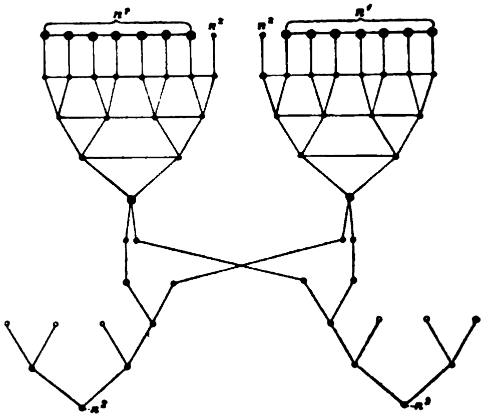
At different times during the winter more conjugates were found, in each case on only one Asterina out of several kept in the same aquarium and examined every two or three days. In every case the host was one that had been kept in the laboratory for from four to six weeks, supplied with fresh sea water every day, but with no food.

Methods. — It was found by experiment that the conjugating pairs, after they had attached themselves to the slide, could be fixed so that they would remain fastened to the glass during the processes of hardening, staining, dehydrating, clearing, and mounting in balsam. The method used was to drain off most of the water and pour on a mixture of absolute alcohol and 5 % glacial acetic acid, or Boveri's picro-acetic. Any fixing fluid containing osmic acid caused the Licnophorae to loosen their hold on the glass, and corrosive-acetic (3 %), though at first apparently successful, resulted in loss of the specimens in the alcohols. MAUPAS's glycerine methods were not successful with this form. The micronucleus of Licnophora is difficult to stain well with anything except iron-haematoxylin, but that can be used with satisfactory results only for sections, and here it was necessary to work with whole material. Several stains were tried but nothing else gave better results than staining about twelve hours with alum-carmine and decoloring under the microscope with acid alcohol. The specimens were then dehydrated, cleared with xylol or clove oil and mounted in balsam.

Stages of conjugation. — On account of the limited number of conjugates found, and the difficulty in distinguishing the faintly staining micronucleus among the many deeply staining macronuclear segments, not all of the stages were obtained, but enough, I think, to show what the formula of micronuclear changes must be for this form.

Figure 30 is a free-hand sketch of the first pair of conjugates seen in material from Thysanozoon. Here the attachment discs are toward the observer, while in all other cases they were attached to the slide with the dorsal surface toward the observer (Figs. 31—39). The gametes are equal in size and are united by the central portion of their ventral surfaces within the peristomal band. The field of union does not include the mouth, but I have never observed any feeding during the period of conjugation. The adoral cilia are usually curved over ventrally so that their tips meet or interlace, as shown in Figs. 31—36, and there is very little ciliary motion after the conjugates are attached to the slide, unless they are disturbed. The oral disc, instead of being flattened dorso-ventrally and somewhat concave on the ventral side, is contracted into a nearly spherical form (Fig. 30). The macronuclear segments lose their regular arrangement, and are variously disposed in the dorsal region, leaving the now convex ventral peristomal field free for the evolutions of the micronucleus.

Figure 31 shows a very early stage where the micronuclei have moved but little from the usual position near the attachment cup. In Fig. 32 we have a slightly later stage with the micronuclei in the ventral region, and in Fig. 33 is shown the only case of micronuclear division observed. The spindles are similar to those seen in fission of Licnophora macfarlandi (Fig. 4). Figure 34 shows the beginning of the second micronuclear division. Figure 35 is a stage in which the third division has occurred and the pronuclei are seen in the ventral region, while the three other micronuclei in each gamete are among the macronuclear segments in the dorsal region. The macronuclear material is more granular in this stage than in the preceding stages. Figure 36 shows the only specimen seen in which preparation for exchange of micronuclear elements was evident, but it proved to be impossible to demonstrate the micronuclei. Figure 37 shows a pair on the point of separating, and Fig. 38 an exconjugate just after separation. In this stage the micronucleus is always in the position indicated, and is always very large. During the separation of the gametes the cilia are again in rapid motion. Figure 39 shows the first division of the conjugation micronucleus, and Figs. 40—43 later stages. Nothing was found between the stage shown in Fig. 39 and that in Fig. 40, where three divisions of the conjugation nucleus have occurred, the eight resulting nuclei remaining connected. At one end of the chain is the micronucleus, of about the usual size; the remaining seven segments are much larger, but have not yet reached the normal size. The old macronuclear segments are only faintly visible. Figure 41 is a similar stage where a few of the old segments are still distinguishable. In these stages the micronucleus is not always found near the attachment cup. Figures 42 and 43 show the macronuclear segments in division, and in Fig. 43 connection between the micronucleus and the macronuclear chain has disappeared.



Textfigur A. Diagram showing the evolutions of the micronucleus of Licnophora during conjugation. n^1 = macronuclear chain, n^2 = micronucleus.

The micronuclear phenomena described may be represented as above, the formula being similar to that for other ciliates, but differing from all other cases recorded in that seven of the nuclear elements resulting from the three divisions of the conjugation nucleus remain connected as the macronuclear chain of the exconjugate.

Physiological considerations. — The conjugation of Licnophora was studied mainly from a morphological point of view. A few physiological points were noted incidentally, but material was lacking for an extended series of experiments, even if that were possible with marine forms. Although, as was stated above, conjugating gametes were found only on animals that had been for some time in the aquaria, the infusoria did not appear to be in a starving or otherwise unhealthy condition. There was no more variation in size than usual, and food masses were present in early stages of conjugation. A few cases of slightly abnormal division were observed: either cell-division was delayed, or it occurred before division of the macronucleus was complete. Figure 19 shows a case of conjugation in which one of the gametes was one of a pair undergoing ordinary division or fission, but they had not separated at so early a stage as usual, as appears from the condition of the macronucleus in the two daughter individuals. Conjugation usually occurred between individuals of approximately the same size and nuclear conditions, — the ordinary adult size before any signs of approaching division appear. Occasionally one or both gametes was larger and showed in the macronuclear segments the separation of the chromatin which is characteristic of early stages of division. Conjugation, therefore, appears to be possible at any time in the cycle between one division and the next.

After the first pair was discovered, many preparations were made by mixing material from different hosts, but no cases of conjugation resulted. All unions were effected before the specimens were transferred from the host to the slide, and may, therefore, have been exogamous or endogamous.

One point of interest is the fact that all of the exconjugates observed were small, emaciated, and free from food masses. This suggested that, at least in Licnophora, conjugation may be an exhausting process, leaving the exconjugates in a weak condition which they may or may not survive, according as circumstances are favorable or unfavorable. If this on further observation should prove to be true, it may account for the fact that Calkins ('02) found that only about 6 % of the exconjugates in his hay cultures survived, while 83 % of the wild exconjugates lived and multiplied, and one endogamous exconjugate, isolated and treated with beef extract, went on dividing up to the three hundred and fiftieth generation.

The real significance of conjugation in Protozoa and its relation, if any, to fertilization in Metazoa are questions of vital interest and much discussed at present.

BÜTSCHLI, ENGELMANN, GRUBER, MAUPAS, HERTWIG, BOVERI and others agree that union of the micronuclei of the two gametes is the essential thing in the process of conjugation, but very different opinions are held as to why conjugation occurs.

MAUPAS ('89) considers that conjugation is a rejuvenating process necessary to prevent senile degeneration and death. BÜTSCHLI ('76) and ENGELMANN (76) have described the object and the result of conjugation as "Verjüngung" and "Reorganization" respectively.

R. HERTWIG ('99), finding that when either in overfed or in starving Actinosphaerium, the nucleus becomes disproportionately large, a reduction in size is accomplished by absorption of a part of the nuclear substance by the cytoplasm, suggests that degeneration of the macronucleus in ciliates, and reduction of the micronucleus in ciliates and of the nucleus in other Protozoa, may have a similar explanation; i. e., reconstruction of the nucleus is rendered necessary by a disproportion between nucleus and cytoplasm. With this interpretation reduction has a physiological rather than a phylogenetic significance.

GRUBER ('86) agrees with WEISMANN that the object of fertilization and of conjugation is to introduce variability of individuals and to build up new species. According to this theory, the micronucleus contains only "Keimplasma", while the macronucleus consists of a very small amount of "Keimplasma" with a large amount of "Histogenicplasma" taken up from the cytoplasm after conjugation and subsequent division of the conjugation nucleus. The macronucleus, in their opinion, controls all the functions of the cell, the micronucleus being active only in conjugation.

CALKINS ('02), as a result of his recent experiments with Paramoecium, maintains that conjugation is not necessary to prevent senile degeneration, since the so-called rejuvenescence may be accomplished by other means. Degeneration and death in his cultures appeared to result from weakened powers of digestion and assimilation; the infusoria were restored to health and reproductive activity by the use of various stimulating agents, notably beef-extract and extract of sheep's brain. On one occasion the infusoria recovered from a period of depression apparently as a result of slight mechanical stimulation. It is to be remembered, however, that the conditions in artificial cultures are quite different from those in nature; and these experiments afford no convincing proof that what Calkins designates as "parthenogenetic" recovery from a period of depression, is in any way comparable to the results of conjugation.

Calkins also finds that no one of Maupas' three conditions, — hunger, diverse ancestry and sexual maturety, are necessary for "fertile" conjugation. Another interesting observation which he made was that in nearly every case one of a pair of exconjugates was more fertile than the other, indicating the possibility of "incipient fertilization". Watasé's suggestion that difference in sex is a temporary differentiation of protoplasm in one of two different directions, is significant in this connection.

It is very evident that much more extended observations of conjugation in many different species of Protozoa under as nearly normal conditions as possible, are necessary before any definite conclusions can be reached as to the whole significance of conjugation in the life history of the Protozoön, and as to the relation between conjugation in Protozoa and the processes of maturation and fertilization in Metazoa.

Regeneration in Licnophora. — While working with Licnophora auerbachii at Naples, it occurred to me that it would be interesting to ascertain whether so highly organized a ciliate as Licnophora would regenerate to the same extent as Stentor. Like Stentor, Licnophora has a segmented macronucleus, making it possible to cut the animal into several pieces each of which contains one or more nuclear segments; but unlike Stentor, this ciliate has but one micronucleus and that in a definite and somewhat isolated position close to the wall of the attachment cup, as shown in Fig. 44. Licnophora also differs from Stentor in having a highly complicated suction apparatus, or attachment disc at the posterior end, and a large ventral peristome, leaving only a small portion of comparatively undifferentiated cytoplasm between the two discs.

Methods. — The Licnophorae were taken from Asterina with a pipette, placed on a glass slide and allowed to attach themselves to the glass. The cutting was done with a sharp lancet needle under a Bausch and Lomb 1 inch objective and ocular Cap. C. The slides were then placed in a moist chamber similar to that described by Maupas ('89) for his experiments with Protozoa; or in some cases the fragments were removed to watch glasses so that the water could be changed frequently, and these were kept in the moist chamber. A few were cut on the paraffined slides used in my experiments on Echinus eggs ('02), but this method did not prove to be as advantageous as cutting on the glass and leaving the animals attached to the slide when possible. In some of the experiments the specimen after the operation was kept under constant observation for several hours, more water being added at intervals to counteract as far as possible the plasmolyzing effect of the evaporating sea water, which makes it more difficult to experiment successfully with marine than with fresh water organisms.

Experiments. — The first set of experiments had for its object to determine whether the oral disc, if removed from the attachment disc, would regenerate a new attachment disc. The animals were cut through the neck region as in Fig. 44, *x . . . x*, leaving the smaller posterior piece, containing the micronucleus and three or four segments of the macronucleus, attached to the glass, and the larger anterior portion, containing from eight to ten macronuclear segments free in the water. The two separated discs behaved quite differently. The attachment disc continued to rotate as usual, but more rapidly for a time. The oral disc contracted and ceased to move for a short time as through paralyzed, then expanded and began to whirl about by means of the usual movements of the peristomal cilia. The attachment disc often lived for several days, but showed no sign of regeneration; that was hardly to be expected, however, on account of the complicated suction apparatus and the small amount of undifferentiated cytoplasm. There appeared to be no reason why the oral disc should not develop a new attachment disc; but, though these pieces lived for from six to nine days, they never showed any indication of regeneration. The cut surface closed quickly, and the pieces lay on the surface of the glass, usually ventral side up, sometimes quiet, at other times whirling about or even turning over repeatedly. There was no evidence that they took any food. After several days they became very transparent and seemed to be in a starved condition though there was plenty of food material at hand; they moved less frequently and soon died. Three possible interpretations of the failure of these pieces to regenerate an attachment disc presented themselves. (1) These ciliates might not be capable of regeneration. (2) Failure to regenerate an attachment disc might be due to the fact that there was no micronucleus present in the oral disc. (3) Licnophora may not be able to form a new attachment disc in any other way than by division of the old disc as in the process of fission.

The next thing to be done was to find out whether regeneration would take place if the animals were cut in some other region.

Several specimens were cut as in Fig. 45, *a*....*a*, *b*....*b*, and left in the moist chamber over night. The small pieces rounded up in various forms (Fig. 46, *a* and *b*), and moved about over the surface of the glass, but no regeneration occurred. The next morning after the operation, the larger part of the animal had closed in at the anterior cut-end, so that the two ends of the oral band were united, and the cytoplasm had shifted so as to restore the usual form of the oral disc. Whether the oral disc and the peristome would later grow to the normal size I was unable to determine. The changes that did occur could hardly be called regeneration, but rather repair and "regulation". Animals cut in this way were watched under the microscope, and seen to close in as in Fig. 47, with the ends of the ciliary band united, but with a notch at the point of union. This notch disappeared later (Fig. 48). One such specimen, cut as in Fig. 45, *c*...*c*, had closed in completely and was feeding after fifteen minutes, making perfectly normal, coördinated movements of its shortened band of cilia, the oral spire being approximately one half of its usual length, and the disc two thirds of its former size. The smaller piece in this case, at first hung by a thread of protoplasm, but was detached after five minutes. The animal twice loosened itself from the slide and finally shook off the piece, as it does any foreign object which may have become entangled in its cilia.

When the line of separation of the two parts passes through the mouth region (Fig. 45, *d*....*d*, *e*....*e*), the two cut ends of the ciliary band do not come together, but new cilia form between the ends (Fig. 49), and later the disc assumes approximately its normal form, but both mouth and peristomal spire are, of course, smaller than usual. Here we have regeneration in the production of new cilia to complete the peristomal ciliary band, and reorganization or shifting of the cytoplasm to bring the mouth and spire into normal relations.

If the cut is made at *f*...*f*, removing the whole, or all but a few rows of the peristomal band, a movement of the cytoplasm from the attachment disc into the neck and the small part of the oral disc remaining, occurs, and a small new oral spire is formed (Fig. 50). This result was also obtained in one case where a small part of the pharynx remained. The new peristome being formed on the ventral side next to the glass, I could not ascertain whether the method of development was the same as in fission or not. When first seen these new peristomes appeared to be complete circles of short cilia, and the intermediate stages between this and the left-turning spiral were not observed. At first these specimens were very short and broad (Fig. 50), but in the course of twenty-four hours the attachment disc grew smaller and the oral disc longer and narrower, giving nearer normal proportions (Figs. 51 and 52). Figure 52 shows the smallest new peristome observed.

When pieces were cut off diagonally (Fig. 45, *g*....*g*, *h*....*h*, *i*....*i*), new cilia were formed to complete the spire (Figs. 54 and 55).

From these experiments it appears that repair, reorganization and regeneration, so as to produce a complete and fairly normal organism, are possible in pieces of Licnophora consisting of the attachment disc, neck, and one fourth or more of the oral disc. The smallest pieces that produced a new peristome were much smaller than the whole oral discs which did not regenerate an attachment disc.

None of the pieces removed from the oral disc showed any sign of regeneration, though they lived and moved about for several days.

Various experiments were made to see whether a cut extending some distance into the oral disc would close. In nearly every case, even when some cytoplasm escaped, the parts came together almost instantly, and in a few minutes no trace of the injury was visible. In some cases the ends of the ciliary band did not meet exactly, but this did not prevent perfectly coördinated movements of the cilia.

The attachment disc was also cut in various ways. Cuts extending from two thirds to three fourths of the distance across the disc usually closed very quickly, and after fifteen to thirty minutes no trace of the cut remained. The attachment disc of such a specimen is shown in Fig. 56. The edges of the cut separated very widely at first (*b*), but came together quickly, and at the end of a minute just a trace of the cut was visible (*c*). In the case shown in Fig. 57, the cut closed quickly, but a notch and traces of the injury remained after twenty minutes; these completely disappeared in the course of four hours. Figure 58 was drawn from a specimen in which the cut closed more slowly from the inner end outward. At the end of five minutes the cut was still open, as in *b*; after thirty minutes it had closed, but imperfectly, leaving the edge of the disc jagged, and the velum not united. Three hours later the irregularity in the outline of the disc was still noticeable.

Cutting off a small portion of the disc gave such results as appear in Figs. 59 and 60. The cytoplasm rounded out at the cut edge, and the ends of the velum came gradually nearer together, giving such appearances as are shown in Figs. 59, *c* and 60, *c*, at the end of fifteen and twenty-five minutes respectively. Perfectly normal discs were observed the next morning in several such cases, indicating slight regeneration in the velum and ciliary membranes. If half or more of the disc was removed, the animal loosened itself from the glass, the mutilated disc was more or less absorbed, and no regeneration occurred.

In one case the pellicula and most of the cytoplasm from the dorsal side of the disc was accidentally removed, leaving the cup, velum and membranes as seen in Fig. 61. The cytoplasm moved forward from the neck region over the exposed cup until at the end of thirty minutes it was completely covered (Figs. 62 and 63) with a thin layer of cytoplasm. Four hours later the outline of the disc remained somewhat irregular, but the animal had resumed its usual rate of rotation which during the first half hour after the injury had been very slow.

When the animals were cut lengthwise in halves, the pieces rounded up and lived for some time, but did not regenerate, though each half must have contained several nuclear segments.

The whole series of experiments shows that regeneration in Licnophora is very limited, being confined to the production of a few new oral cilia, a new peristome and possibly a very small portion of the attachment disc. The organism, however, possesses marked powers of repair and "regulation" in the sense used by DRIESCH. Further experimentation, together with histological study of the regenerating pieces, is necessary in order to determine whether regeneration in this form is in any way dependent on the presence of the micronucleus, as the failure of the isolated oral disc to regenerate a new attachment disc suggested. Since the formation of new parts in Protozoa usually follows the same method as the development of those parts in the process of fission, and the new attachment discs are formed by elongation and division of the old disc, and not independently as is the new peristome, it seems probable that Licnophora is incapable of regenerating an attachment disc; but further study of this and related forms is necessary to prove this point.

In connection with these experiments a curious case was noticed where a specimen, otherwise apparently normal, had a second oral disc, somewhat smaller and attached to the left dorsal side. This second disc sometimes lay with its ventral side against the dorsal surface of the larger disc as in Fig. 64; but, when feeding, the disc was thrown out exposing the peristome as in Fig. 65. It was first seen on Feb. 28th; on March 1st both discs were feeding normally; but on the following morning the abnormal disc appeared to be growing smaller, the cilia disappeared, and the disc seemed to be sinking into the normal disc. At noon it was reduced to less than half its former size. On killing the specimen with aceto-carmine, it was found that the macronuclear segments of the attachment disc were in normal position, but there were only three segments in the peristomal group, and two of those were in the abnormal part (Fig. 66). The position of this secondary disc did not indicate an abnormal form of fission, nor was there any possibility of its being a case of conjugation of unequal gametes. The most probable explanation seemed to be that it was an abnormal growth due to injury.

The literature on regeneration in Protozoa deals mainly with the bearing of the experiments on the functions of the nucleus. (EICHHORN 1783; HAECKEL '68; GREEF '67; BRANDT '77; NUSSBAUM '84; GRUBER '85, '86 and '87; VERWORN '88 and '91; BALBIANI '88 and '92—93; HOFER '89; LILLIE '96; MORGAN '01.) No experiments on the Peritrichae are recorded. GRUBER alone mentions the micronucleus in connection with regeneration, expressing the opinion that it plays no active part in the process. He cites as evidence that it is the macronucleus and not the micronucleus that is essential in regeneration, the fact that pieces of conjugating Stentors do not regenerate until a stage is reached in which one of the micronuclei has assumed the form and functions of a macronucleus. These experiments are not convincing, however, since the micronucleus, in the stages of conjugation in which regeneration does not occur, is undergoing such a series of changes connected with the phenomena of conjugation that it could hardly be expected to take part at the same time in another process.

Reaction of Licnophora to electrical stimulus. — Three years ago at Pacific Grove, I tried the effect of the constant current on Licnophora, and the few observations made then promised interesting results; but the apparatus at hand was not suited to the work. At Naples it was possible to continue the experiments and obtain definite results.

Methods. — The apparatus used was a battery for physicians, combining in one instrument a battery of thirty elements, induction apparatus, rheostat, indicator, and keys for shifting the current and for turning it on or off, as desired. This instrument, manufactured by REINIGER, GIBBERT and SCHALL, of Erlangen, is the most convenient piece of apparatus for such experiments that I have seen, provided that a strength of not more than 30 M. A. is required.

Pieces of filter paper about 1,5 cm square were placed on a wide glass slide, leaving a space of from 0,5 cm to 0,7 cm between them, and on these rested non-polarizable clay electrodes. The water containing the Licnophorae was placed on the slide with a dropper, the animals allowed to attach themselves, and a favorable individual brought to the center of the field of the microscope before the electrodes were adjusted and the current turned on. Observations were made with a Zeiss A objective and ocular 4; no definite results could be obtained with a cover-glass and a high power.

Normal movements. — In connection with the following experiments, it was found necessary to make a careful analysis of the normal movements of Licnophora for comparison with the movements noted when the organism was subjected to the action of the current.

A. Movements when the animal is attached: —

1. Slow rotation on the longitudinal axis, effected by the ciliary membranes beating against the slide or any other surface.
    - (a) Oral cilia moving with the effective stroke toward the mouth.
    - (b) Oral cilia suddenly clapped down on the ventral surface sending a current toward the mouth.
    - (c) Oral cilia at rest except in the mouth and pharynx.
    - (d) Oral cilia beating either ventrally or dorsally.
    - (e) Oral disc bending dorso-ventrally or ventro-dorsally through an arc of 120° or more, with the oral cilia at rest, the bend being made at the neck.
2. No rotation, ciliary membranes and velum not vibrating.
    - (a) Oral cilia at rest except in the mouth and pharynx.
    - (b) Oral cilia sending food currents to the mouth as in *a* and *b* above.
    - (c) Oral disc turning now this way now, that by bending or twisting the neck.

B. Swimming movements: —

1. Forward movement for a short distance, probably effected by the ciliary membranes beating against the water in the same manner as against a surface; rotation from left to right as when attached.

2. Whirling round and round, the two discs in the same plane, due to the action of the oral cilia turning the animal toward the aboral side.
3. Turning in various directions according to the comparative violence of the vibration of the cilia of the two discs, and their relative position.

Experiments and observations. — In general the orientation of Licnophora by the constant current was as follows: —

A. Attached to the slide.
  1. Aboral side toward the anode and oral disc at right angles to the slide (Fig. 27).
  2. Violent vibration of the oral cilia on the anode side and a turn of 180° at each change of the current (Fig. 28).

B. Swimming: —
  1. Movement toward the kathode, oral disc forward, often with many turns, but sometimes straight across the field, whirling on the longitudinal axis, as when attached or when swimming without the influence of the electric current.
  2. Stimulation of the oral cilia on the anode side, longitudinal turn through 180°, and swimming toward the kathode, at each change of the current.

Effective current. — For most individuals a current of about 20 M. A. was necessary to orient them when they were fresh from the host and very active; for a few 25 M. A. was required, and after an individual had been experimented with for an hour or more, 10 M. A. was sufficient, and in one case 9 M. A. A few examples are given below.

I.

Two fresh specimens.

20 M. A. effective for one.
25 M. A. effective for the other.

II.

Fresh specimen.

9 M. A.	0[1]
10 „ „	0
15 „ „	0

III.

Fresh specimen.

20 M. A.	0
25 „ „	+
15 „ „	+ (after 15')

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[1] 0 = not effective. + = effective.

20 M. A.	+	10 M. A.	0
17 „ „	+	12 „ „	+ (after 30')
16 „ „	+	11 „ „	+ („ 40')
15 „ „	0	10 „ „	+ („ 60').

Individual variations. — Some specimens turn through 180° at each change of the current and remain at rest in the position shown in Figs. 27 and 28, with the oral disc at right angles to the slide and to the direction of the current, aboral side toward the anode, as though held there by the force of the current, the reaction being the same for any effective strength of current. Some, after turning to the position shown in the figures, turn back 40°—50°, then forward again and repeat these movements until the current is changed, when they immediately turn 180° and go through the same backward and forward movements.

One case was observed where the oral disc was held nearly parallel to the slide as in Fig. 29. A current of 20 M. A. caused the animal to come to rest with the anterior end toward the kathode. With a current of 25 M. A. it swung around 15°—20° to the position $b^1 \dots b^2$, but always came back to $a^1 \dots\dots a^2$. After an hour, 10 M. A. was sufficient to prevent it from turning beyond a^2; it swung back and forward between a^1 and a^2, remaining most of the time in the quadrant $a^1 \dots\dots a^1$. With 8 M. A. it succeeded in turning completely around after several trials, but was very slow in responding to a change in the current when it was at rest.

In every case the oral cilia on the side toward the new anode beat violently the instant the current was changed, but in some cases, after turning 45°—50°, these cilia all came to rest, and still the animal turned through the remainder of the 180° by the action of its ciliary membranes against the glass, and frequently rotated backward and forward through a quadrant or more. A dorso-ventral bending of the neck carrying the oral disc through an arc of 45°—50° was also observed while the oral cilia were at rest and the animal was swinging through a quadrant, but not beyond the position shown in Figs. 27 and 28.

In some cases there seemed to be a violent muscular effort, when the ciliary membranes were at rest, to turn the oral disc beyond the critical point, but without effect. This movement is identical with normal movements of the oral disc in feeding when the attachment disc is not rotating, but is held fast to the host by the attachment apparatus (A. 2, *b* [1]).

The rotation on the longitudinal axis of the animal is an almost constant normal movement, and is easily brought about, when not occurring, by such a slight mechanical stimulus as jarring of the slide or by a current of water. The special action of the electric current seems to be confined to orientation in a certain direction, — the longer axis in line with the current, the aboral side toward the anode and the mouth opening toward the kathode. The ciliary membranes seem to be powerless to turn the oral disc beyond a certain point. After reaching this point, their action may be reversed so as to turn the disc backward, a movement which I have not been able to detect under ordinary conditions, but noticed once in a case where an individual entangled in some debris on the slide was trying to free itself.

The response to the current, both when attached and when swimming, is complicated by the ordinary feeding movements of the oral cilia, and by the various turns and twists of the neck; but it seems to me that all of these movements may be disregarded and the effect of the current, when the oral cilia are at rest and the position of the oral disc relative to the attachment disc is constant, may be regarded as the true response. The vibration of the oral cilia on the anode side, too, seems to have no necessary relation to the change in the position of the animal occasioned by a change of the current; it is probably an expression of the first effect of the changed current on the anode side of the organism. The fact that the ciliary membranes beat in a manner to produce the usual rotation, and when that proves ineffective, beat so as to rotate the body backward and then forward again, shows that the membranes are not held to any forced position by the current, but that, in the last analysis the effect of the current is to hold the body in a certain position relative to the current. This position is apparently determined by the form and structure of the body, since the long axis of a horizontal section is always in line with the current, and the mouth opening toward the kathode.

When the animal is swimming, it is difficult to tell whether the sudden longitudinal turning through 180° at each change of the current is due wholly to the action of the stimulated cilia on the anode side or in part to muscular action. Orientation is always as in Fig. 29, and the action of the oral cilia and ciliary membranes is the same as in ordinary swimming except that movement in a definite direction is long continued, while ordinarily the animal darts hither and thither, whirling and turning in every direction. Here, too, the effect of the current seems to be limited to orientation of the organism with the long axis in line with the current, and the anterior end and mouth opening toward the kathode, so that the animal continues to swim in one direction by means of the ordinary action of its ciliary membranes.

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[1]) page 29.

As in the case of Amoeba, Actinosphaerium, Paramoecium and other ciliates, and of flagellates [recently shown by Pearl ('00)], Licnophora under the action of the constant current swims toward the kathode. Comparison of the behavior of Licnophora under electrical stimulation when attached and when swimming, with its movements when not subjected to the action of the electrical current, leads me to think that orientation in both cases is connected rather with bodily form and structure than with any special effect on the cilia, causing them to take "forced positions" as described by Pearl ('00) for Paramoecium and other ciliates. The responses of Licnophora are especially interesting on account of its structural peculiarities, localized ciliary apparatus, and the fact that without change in structure it may be either attached or free-swimming.

Few if any experiments of this kind upon peritrichous Infusoria have been described. The simpler forms of Protozoa have been used for experiments in galvanotaxis, and the attention of investigators has been directed mainly to orientation, to changes in the form of the body, and to the action of the cilia. (Kühne '64; Verworn '97; Ludloff '95; Jennings '97; Loeb and Budgett '97; Birnkoff '99; Carlgren '00; Pearl '00.) For comparison with the results obtained with Licnophora, experiments should be made with other highly differentiated ciliates, with permanently fixed forms, and with other forms which may be either fixed or free-swimming, as Trichodina, Stentor and Boveria.

Boveria.

A short time before I left Naples, I learned that a species of Boveria, apparently identical with Boveria subcylindrica (Stevens '01), occurs in abundance on the bivalves, Tellina exigua and Capsa fragilis. I obtained the material and was able to clear up several doubtful points in my previous work, notably the formation of the oral spire in fission, and the number of chromosomes that appear in divison of the micronucleus. Doubtless Boveria occurs on other hosts in the Bay of Naples, but I did not have time to investigate further. No cases of conjugation were observed, but it may be possible to obtain them by keeping the hosts in the laboratory several weeks as was done with Licnophora.

Comparison of the Monterey and Naples forms. — To summarize briefly the description of Boveria subcylindrica given in full in my "Studies on Ciliate Infusoria" ('01), this species is characterized by an elongated body varying from a cylindrical form to that of a truncated cone with rounded ends. The length is from 54 μ to 81 μ, the width at the oral end 18 μ to 21 μ, and at the aboral end 9 μ to 18 μ. The terminal oral spire consists of a double row of long rather coarse cilia, making one complete turn and about 290° of a second turn. The mouth is within the loop formed by the union of the two rows of oral cilia at the inner end of the spiral (Figs. 67—69, and '01, Pl. IV and Pl. V). The whole surface of the body, with the exception of the peristome and a small circle at the aboral end is covered with longitudinal rows of fine cilia, much shorter than the oral cilia. Just posterior to the peristome and about 100° from the onter end of the ciliary spiral is a large contractile vacuole with a variable period averaging from two to three minutes (Figs. 67 and 69, *v*). The nucleus, which is faintly visible in the living animal, is a large oval structure, centrally placed, and showing in sections a linin network, coarse chromatin granules, and often from two to four large nucleoli ('01, Pl. V, Figs. 48, 53, 54). Near the aboral end is a large micronucleus which stains deeply with iron-haematoxylin, safranine, carmine, methyl green, and other nuclear stains. Between the micronucleus and the aboral end is a lenticular disc of very dense cytoplasm, observable in the living animal and in all preparations (Fig. 69).

Boveria is, when undisturbed, essentially an attached form. In the respiratory organs of Holothuria, it appears to hold itself against its host by the constant motion of the body cilia with the effective stroke toward the peristome, but in live cultures it is frequently seen attached by the tips of its aboral cilia to the glass or to other objects on the slide (Figs. 67 and 68). When disturbed, Boveria swims very rapidly in a slightly serpentine course, aboral end forward, with a slow rotary motion and slight flexions of the oral end of the body.

Division in Boveria is of the variety known as oblique fission ('01, Pl. IV, Figs. 38—45). The peristome and contractile vacuole disappear and new ones are formed in the two daughter animals. The micronucleus divides first, and the two resulting micronuclei take positions at the two ends of the elongating macronucleus, which then divides without the formation of a distinct spindle or chromosomes. In the division of the micronucleus, a peculiar series of stages was observed in sections, and two cases are figured where distinct chromosomes were seen at each pole of the micronuclear spindle ('01, Pl. V, Figs. 57, 58, 67); but the number of cases of division where the chromosomes were clearly seen was too small for any definite conclusions.

The Neapolitan Boveria differs but little from the above description, in structure, form or movements. The specimens found on Tellina varied in length from 37 μ to 102 μ, and those on Capsa from 65 μ to 121 μ, some of the latter being longer than the Licnophorae on the same host. The longest individuals are considerably longer and more slender than those whose nuclei indicate approaching division, and the difference in length between the youngest individuals and the longest is much greater than in the Monterey form. Evidently there must be a change in proportion just before division.

Figure 73 shows one of the long slender individuals drawn to scale, 116 μ long, 20.9 μ wide at the oral end and 13.95 μ wide midway between the two ends. In Fig. 72 a specimen whose micronucleus shows the first indications of division, is represented, drawn to the same scale. Figures 70, *a* and *b* show young Boveriae just after division. Comparing these figures with Figs. 67—69, it will be seen that the aboral end of the Monterey form is rounded, while that of the Naples form is pointed. The peristome of the two forms differs only in the length of the spire, that of the Naples variety being from 80° to 90° shorter (Fig. 74, *a* and *b*). The contractile vacuole, however, is in about the same position relative to the mouth, and therefore nearer to the outer end of the spire in the Naples form. The arrangement of the cilia, and the structure of cytoplasm, nucleus, and micronucleus seem to be identical in the two forms; but the denser lenticular disc of cytoplasm near the aboral end is not found in the Naples variety (Figs. 69 and 73). Division phenomena in the two forms are the same. Conjugation has not been observed.

Formation of the oral spire. — In the Monterey form of Boveria, it was noted that the peristomal cilia disappear in the early stages of division, and that a new ciliary spire is formed for each individual in the later stages before they separate. The manner in which the new peristomal band develops was not observed, but can now be described for the Naples variety.

The peristomal cilia first appear on the side as a straight band (Fig. 75), which gradually assumes a terminal position, beginning to coil at the distal end (Figs. 76—78). The earliest stage in which I have been able to detect the lateral ciliary band is shown in Fig. 75, where the cilia are hardly longer than the ordinary body cilia, which are omitted in the figure for the sake of clearness. A slightly later stage is shown in Fig. 76, where the distal end of the band has begun to coil, but the cilia are still short, and the two rows are not distinct. Later stages are shown in Figs. 77 and 78. When the two individuals separate, the peristome is usually still somewhat oblique; the outer end of the spire still having a lateral position (Fig. 70, *a*). In the largest adult specimens, an obliquity in exactly the opposite direction is very often noticeable, the outer end of the spire being considerably elevated above the mouth region (Figs. 68 and 73). The method of development of the peristome as a lateral band may ultimately have some bearing on the classification of Boveria, which is at present not settled.

Division of the nuclei in the Naples variety. — Division of the micronucleus is a point of special interest, as Boveria is the only ciliate yet studied which has been found to have a very small number of clearly defined chromosomes in the micronuclear spindle. Figures 79—86 show in outline the principal stages of micronuclear and macronuclear division, and Figs. 87—96 micronuclear division in greater detail. The position of the micronucleus when it first shows signs of division varies from the usual position near the aboral end to a position in contact with the macronuclear membrane (Fig. 79). The spindle usually appears at one side, but near the posterior end of the macronucleus (Fig. 80); in later stages it stretches along the nuclear membrane with its poles approaching the ends of the macronucleus; and the two micronuclei when separated are located at or very near the poles of the dividing macronucleus (Figs. 81—86).

The micronucleus before dividing increases in size to about three or four times its original volume (Figs. 87—89), a notch appears in one side (Figs. 72 and 90), and later the partly separated halves are divided by clefts at right angles to the first division (Fig. 91). The four quarters of the micronucleus lengthen greatly (Figs. 92 and 93), and divide transversely (Fig. 94). The two groups of four chromosomes each then separate, each pair of chromosomes remaining connected by a single fibre (Fig. 96). In some cases the whole spindle must move forward toward the oral end of the animal, and in others the anterior end of the spindle must move further than the posterior end, for the two micronuclei eventually in all cases reach positions near the poles of the macronucleus. The latest stage observed in which the separate chromosomes were evident is shown in Fig. 95. The indications are that the four chromosomes unite as quarters of a sphere, and possibly we have here a demonstration of individuality of chromosomes, as well as a case of a central spindle formed from material derived from the dividing chromosomes.

This micronucleus stains very clearly in all stages with SCHNEIDER's aceto-carmine, and if the infusorian can be found in conjugation in sufficient numbers, it ought to throw some light on the question of maturation, or reduction of chromatin in Protozoa. Unfortunately, I have as yet never seen the conjugation of this form, and did not have time to experiment with it before leaving Naples. I hope, however, to have an opportunity soon to study the phenomena of conjugation in Boveria either at Pacific Grove or at Naples.

The method of division of the macronucleus is shown a little more clearly by the aceto-carmine preparations (Figs. 79—86) than by my earlier figures. At first the macronucleus becomes much longer and the chromatin appears to be considerably increased in amount (Figs. 79—80). Soon a separation of the granules into a central sphere and two elongated polar masses occurs (Figs. 81—82). As the nucleus lengthens, fibres appear between the central and the polar groups of granules (Fig. 83); the central group lengthens and divides into two (Fig. 84). Figure 85 is a later stage where the two nuclei are still connected by the nuclear membrane: the two groups of granules in each nucleus are still distinct; but in a slightly later stage (Fig. 86), no separation of the granules can be detected. In all stages the granules of the polar and central groups appeared to be of the same form and staining quality, the only difference being that in the central group they were more densely packed together. It is possible that some such division center exists here as that figured by SCHAUDINN ('94) for Amoeba crystalligera, GRUBER, and that it owes its origin to the large nucleoli sometimes seen in the resting nucleus ('01, Figs. 54—55); but, if this is so, it is obscured by a covering of chromatin granules. No division center was discovered in the micronucleus.

The apparent interrelation between the micronucleus and the macronucleus of Boveria in division stages recalls again the question of homology between the macronucleus and micronucleus of ciliates, and the nucleus and division center of the Metazoa.

In the discussion of the centrosome question participated in by BÜTSCHLI ('92), HEIDENHAIN ('94), R. HERTWIG ('95, '98, '99), LAUTERBORN ('95, '96), BOVERI ('95, '00), and ISHIKAWA ('00), BOVERI has shown conclusively that the micronucleus of ciliates cannot be homologized with the centrosome of Metazoa; but the behavior of the micronucleus of Boveria still indicates that it must have some influence over the macronucleus during division. There seems to be no other explanation for the constant position of the two micronuclei at the poles of the dividing macronucleus, when they might reach the usual position in the aboral end of each individual by a much shorter path. In Licnophora also, the micronucleus comes into close relations with the macronucleus during division, and the conditions in Kentrochona (ROMPEL [1]) '94) very closely resemble those in Boveria.

Classification. — In my earlier paper on Boveria, I placed it in the order Heterotricha, but was unable to determine the family. If it is to remain there, the limits of the order must be extended to include a form which has long oral cilia instead of membranellae. (BÜTSCHLI '89; LANG '01; CALKINS '01.) The method of formation of the new peristome as a lateral band might indicate relationship to the Stentors; but the oral spire in Boveria is a right-turning one, the nuclei are very different from those of Stentor, no myonemes are present, nor are there body cilia within the peristomal field. The very pronounced band of oral cilia, and the absence of trichocysts prevents this form from being placed under the Holotrichae, and the presence of body cilia separates it from the Peritrichae, which it resembles more closely in the structure of its peristome.

Leaving the question of order and family open for the present, I add the following genus and species descriptions, making the form found at Naples a variety of Boveria subcylindrica: —

Boveria (n. gen. STEVENS '01). — Size variable, 80 μ—120 μ for adults. Form cylindrical or tapering, several times longer than broad. Cilia of two kinds: (1) a general body system of fine cilia arranged in slightly curved longitudinal rows; (2) a terminal peristomal spiral of long coarse cilia in a double row, closed at both ends, and opening out at the inner end to enclose the mouth. Macronucleus oval, central; micronucleus nearer the aboral end. Contractile vacuole near the peristome. Reproduction by oblique fission. Marine forms, parasitic or commensal, usually attached by the cilia of the aboral end.

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[1]) ROMPEL describes the two bodies at the poles of the macronuclear spindle as centrosomes; but both BALBIANI ('95) and HERTWIG ('95) regard them as micronuclei.

B. subcylindrica. — Length of individuals varying from 54 μ just after a division to 81 μ just before division. Oral cilia about one half the length of the body; body cilia one third or one fourth as long, somewhat shorter on the aboral end than on the sides. Aboral end rounded. A disc of denser cytoplasm between the micronucleus and the aboral end. Oral spire consisting of one turn and 290° of a second turn. Found in te respiratory tree of Holothuria californica in Monterey Bay, California.

B. subcylindrica, var. neapolitana. — Length from 37 μ to 116 μ. Aboral end pointed. No disc of denser cytoplasm near the aboral end. Oral spire consisting of one turn and 210° of a second turn. Found on Tellina exigua and Capsa fragilis in the Bay of Naples.

Summary.

1. The European and American forms of Licnophora closely resemble one another in nearly all structural characters.
2. These forms are to be classified, mainly on the basis of macronuclear differences, under three species, — L. conklini, L. auerbachii Cohn, and L. macfarlandi Stevens.
3. Conjugation of equal gametes occurs, resulting in one micronucleus and a macronuclear chain of seven segments in Licnophora auerbachii.
4. The exconjugates are small and emaciated, indicating that conjugation is an exhausting process.
5. Regeneration in Licnophora is limited to a small part of the attachment disc, a part of the oral ciliary band, or a new peristome.
6. All of the pieces that regenerated contained both macronuclear segments and the micronucleus, while the whole oral disc or parts of it, containing several macronuclear segments but not the micronucleus, did not regenerate.
7. The failure of Licnophora to regenerate an attachment disc is probably due to inability to form such a disc in any other way than by equal division of the old disc.
8. Orientation of Licnophora by the constant current appears to be more closely connected with bodily form and structure than

with any special effect on the cilia causing them to take "forced positions".

9. The Naples form of Boveria appears to be only a variety of the species described as B. subcylindrica.

10. The new peristome in Boveria is first a lateral band, which gradually assumes a spiral form and a terminal position.

11. The micronucleus of Boveria has four distinct chromosomes in division stages.

12. The position of the micronuclei indicates some influence over macronuclear division.

In conclusion I desire to express my gratitude to the "Association for maintaining the American Woman's Table at the Zoological Station at Naples and for promoting Scientific Research among Women" for the use of tables both at the Marine Biological Laboratory, Woods Hole, and at the Zoological Station, Naples; also to thank the staff of both laboratories for many courtesies and for untiring effort to secure for me the desired material. I am also much indebted to Prof. Morgan and Prof. Warren for valuable criticism.

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Explanation of Figures.

Plate I.

Fig. 1. Freehand sketch of living Licnophora macfarlandi, ventral view, showing attachment disc and cup (*a*), neck (*b*), oral disc (*c*), point of meeting of velum und vibratile membrane (*d*), vibratile membrane (*e*), oral cilia (*g*), ventral furrow (*h*), basal bands of the oral cilia (*i*), food masses (*f*), ciliary membranes (m^1, m^2, m^3, m^4), mouth (*o*), pharynx (p), and velum (v^1, v^2).

Fig. 2. Similar sketch of Licnophora auerbachii from Asterina, showing the different form of the attachment disc, shorter neck, and absence of the vibratile membrane (*e*).

Fig. 3. Reconstruction from macerations of Licnophora auerbachii, showing the relation of the fibres (f^1, f^2) to the attachment cup and oral spire. n^2 = micronucleus. *b* = basal bodies of the ciliary membranes. *c* = basal bands of the peristomal cilia. *d* = connecting fibres between the fibre (f^1) and the ends of the basal bands (*c*).

Fig. 4. Outline drawing from a whole mount of Licnophora macfarlandi to show the micronuclear spindle (n^2). n^1 = macronucleus. *c* = the new peristome. *s* = micronuclear spindle more highly magnified. Bausch and Lomb objective $^1/_5$, oc. C, camera. *s* was drawn with oil immersion $^1/_{12}$, oc. C.

Plate II (Division).

Fig. 5. Outline drawing of Licnophora conklini, adult form, showing macronuclear segments (n^1), micronucleus (n^2), outer ciliary membrane (m^1), and oral cilia (s^1). B. and L. obj. $^1/_{12}$, oc. A, camera.

Fig. 6. An early division stage of the same species, showing enlarged micronucleus (n^2), separation of chromatin in the macronuclear segments (n^1), and the beginning of a new peristome. Same magnification.

Figs. 7—10. Later stages showing division of the nuclei (n^1, n^2), change in form, and development of a new peristome. Same magnification.

Figs. 11—15. Young Licnophora conklini, showing resegmentation of the macronucleus. Same magnification.

Fixation with alsolute acetic (5%).
Staining with alum carmine.

Plate III.

Figs. 16—18. Camera drawings of Licnophora macfarlandi (Fig. 16), L. conklini (Fig. 17), and L. auerbachii Fig. 18), to show the segmentation of the macronucleus and the grouping of the segments. B. and L. obj. $^1/_5$, oc. C.

Fig. 19. Conjugation between one gamete whose nuclear segments show approaching division, and another gamete not yet separated from its sister Licnophora. Zeiss obj. D, oc. 6.

Fig. 20. Macronuclear segment, showing separation of chromatin and two nucleoli. B. and L. obj. $^1/_{12}$, oc. C.

Fig. 21. Licnophora auerbachii, showing separation of the macronucleus into three parts in the early stages of segmentation. B. and L. obj. $^1/_5$, oc. C.

Fig. 22. Licnophora conklini, showing the macronucleus segmented irregularly and to an unusual extent. B. and L. obj. $^1/_{12}$, oc. A.

Fig. 23. Young L. conklini, next stage after Fig. 15, Pl. II. Same magnification.

Figs. 24—26. Attachment discs of L. auerbachii from Asterina (Fig. 24), Capsa (Fig. 25), and Thysanozoon (Fig. 26), showing difference in form of the disc (*t*), velum (v^1, v^2), attachment cup (*a*), attachment of the neck to the disc (*b*), and notch in the disc (*d*).

Figs. 27—29. Sketches of L. auerbachii under the influence of the constant electric current. $+$ = anode. $-$ = kathode.

Plate IV (Conjugation).

Fig. 30. Sketch of a living pair of conjugates, L. auerbachii from Thysanozoon.

Figs. 31—39. Various stages in the conjugation of L. auerbachii from Asterina, showing macronuclear and micronuclear changes. n^1 = macronucleus. n^2 = micronucleus. *a* in Fig. 35 = degenerating micronuclei. *x* in Fig. 36 = region of micronuclear exchange, pronuclei not visible. *a* in Fig. 37 = conjugation nucleus. Zeiss obj. D, oc. 6.

Figs. 40—43. Exconjugates, showing degeneration of the old macronucleus, and development of new macronuclear segments. Same magnification.

Plate V (Regeneration).

Fig. 44. Diagrammatic drawing of L. auerbachii to show the relative position of the macronuclear segments (n^1), the micronucleus (n^2), the mouth (*o*) and the neck fibre (f^1). $x \ldots . x$ = the line of cutting in the first experiments,

Fig. 45. Dorsal view of L. auerbachii showing how the specimens were cut in the various experiments.

Fig. 46. Small pieces from the anterior end of the oral disc.

Figs. 47—48. Results from cutting at *b . . . b*.

Fig. 49. Result from cutting at *d . . . d*; new cilia at *c*.

Figs. 50—53. Individuals with new peristomes, resulting from cutting at *f . . . . f*.

Fig. 54. Result of cutting at *g . . . . g*; new cilia at *c*.

Fig. 55. Result from cutting at *i . . . . i*; new cilia at *c*.

Figs. 56—60. Results from cutting the attachment disc. Neck and oral disc not shown in the sketches.

Figs. 61—63. A case where the pellicula and cytoplasm were removed from the dorsal side of the attachment disc posterior to *x . . . . x*. Injury repaired by shifting of cytoplasm from the neck region.

Figs. 64—66. Abnormal specimen with a second oral disc (c^2). Fig. 64 with ventral surface of the second disc (c^2) against the dorsal surface of the normal disc (c^1); Fig. 65, ventral surface exposed; Fig. 66 specimen killed and stained with aceto-carmine after partial absorption of the abnormal disc, to show the nuclear conditions.

Plate VI (Boveria).

Figs. 67—69. Sketches of Boveria subcylindrica from Holothuria, showing mouth (*m*), vacuole (*v*), attachment of aboral cilia (*c*), food mass (*f*), macronucleus (n^1), micronucleus (n^2), and denser stratum of cytoplasm near the aboral end (*a*). Figs. 67 and 68 were from living specimens, and Fig. 69 from a section. Hermann fixation, iron-haematoxylin staining, B. and L. obj. $^1/_5$, oc. C.

Fig. 70. Young Boveriae, Naples variety.

Figs. 71—73. Adult Boveriae, Naples variety.

Fig. 74. Diagrams of the peristomal spirals of the Monterey variety (*a*), and of the Naples variety (*b*).

Figs. 75—78. Sketches from living specimens, showing formation of new peristomes. Body cilia omitted for the sake of clearness.

Figs. 79—86. Division stages from the Naples variety. Fixation and staining with Schneider's aceto-carmine. B. and L. obj. $^1/_5$, oc. C.

Figs. 87—96. Division stages of the micronucleus from aceto-carmine preparations. B. and L. obj. $^1/_{12}$, oc. C.

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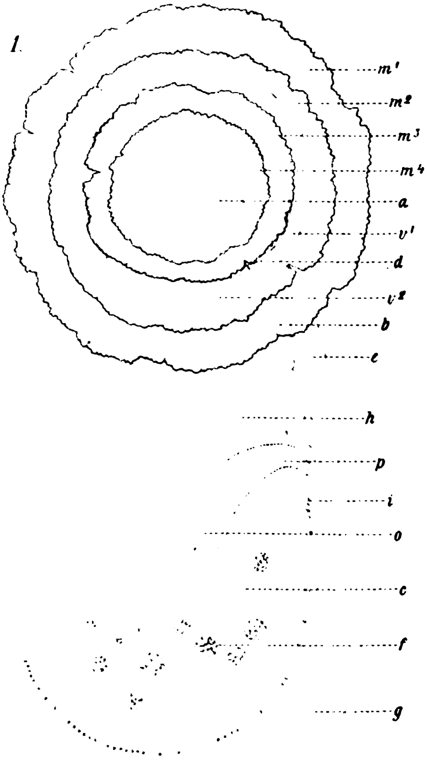

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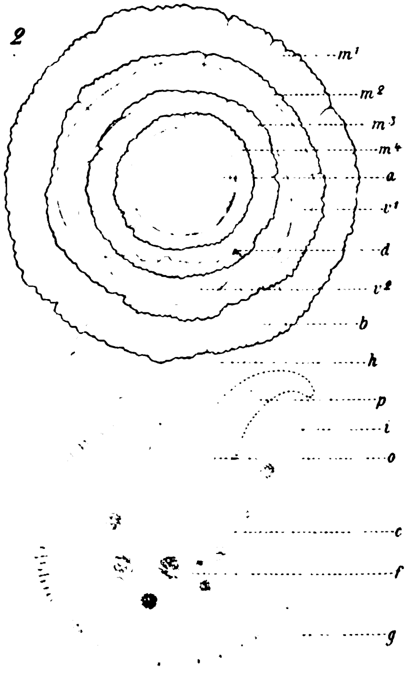

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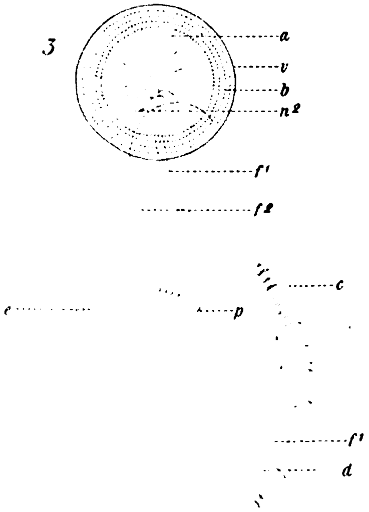

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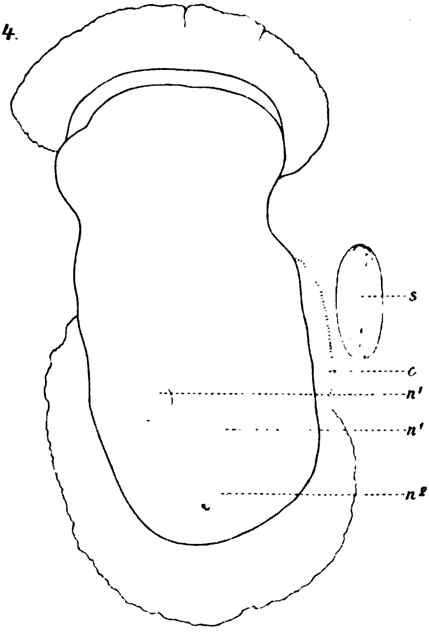

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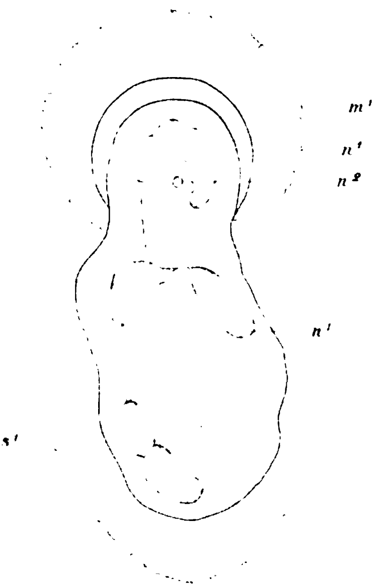

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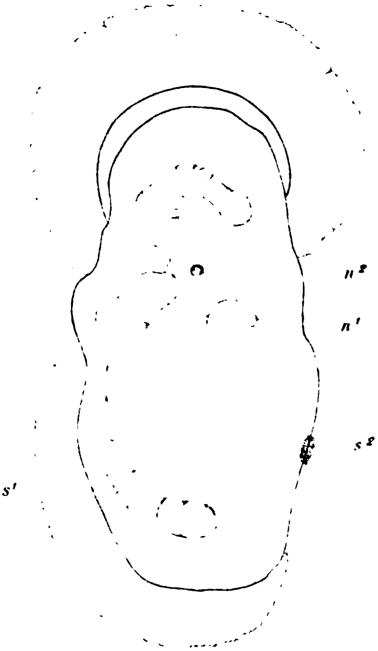

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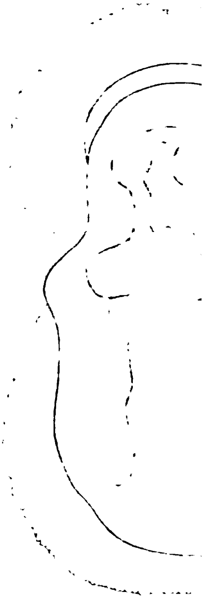

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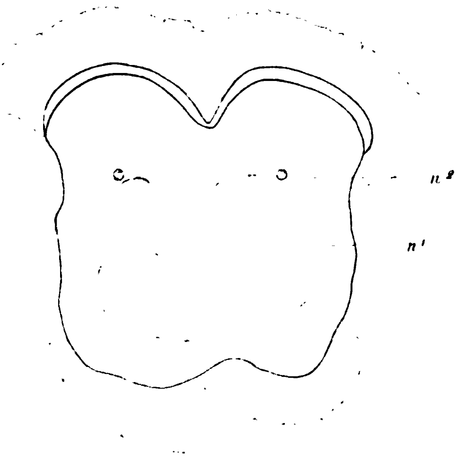

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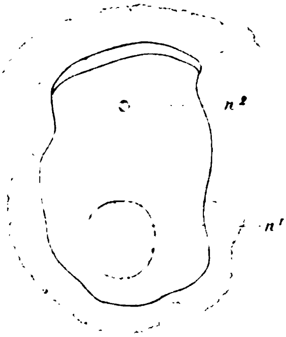

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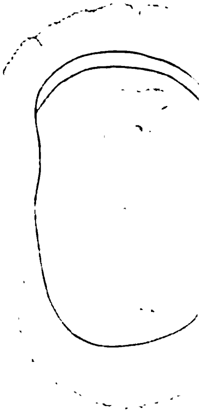

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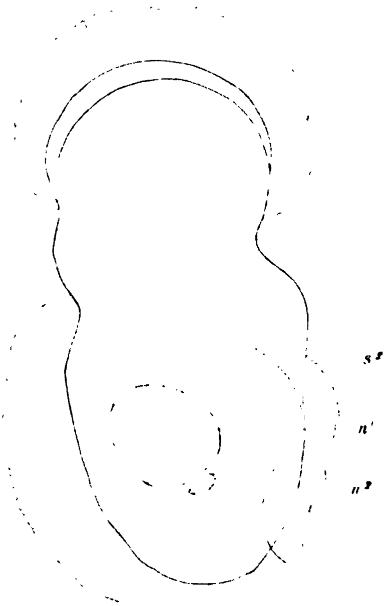

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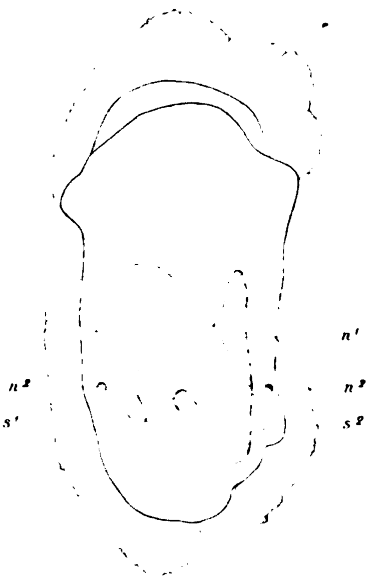

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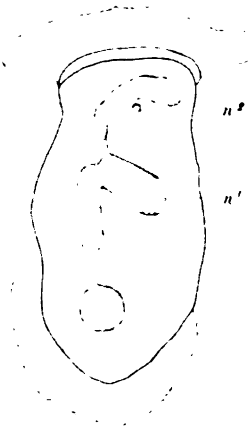

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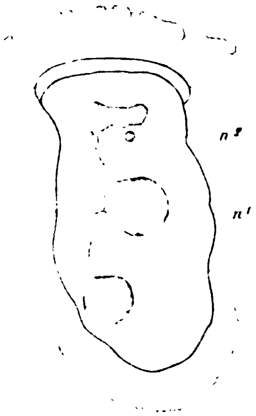

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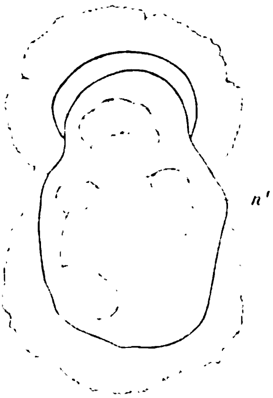

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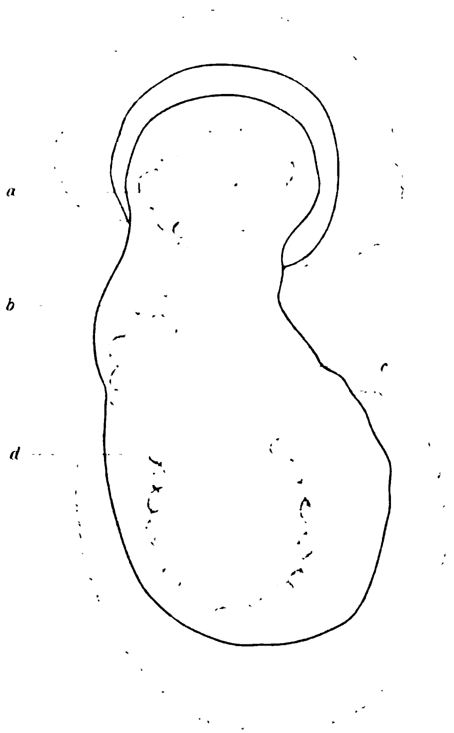

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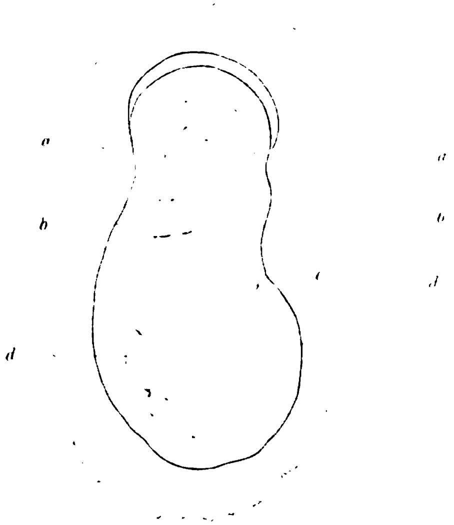

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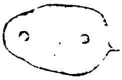

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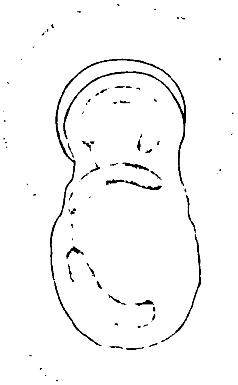

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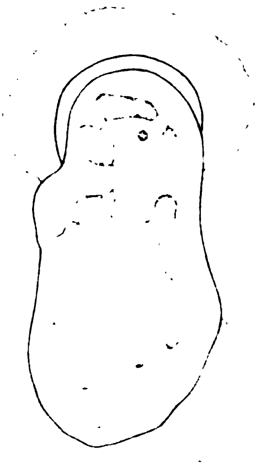

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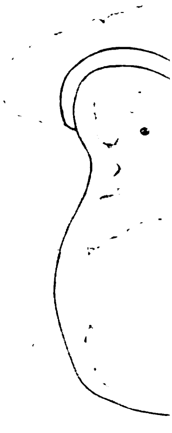

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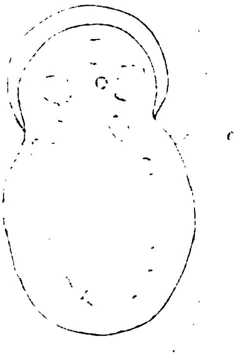

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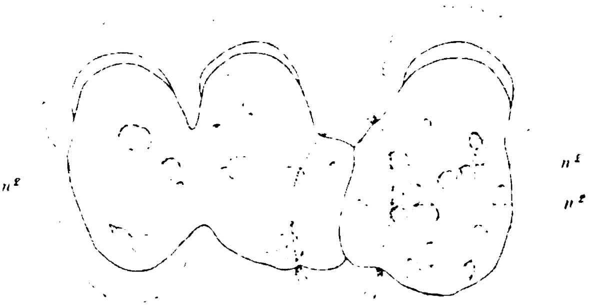

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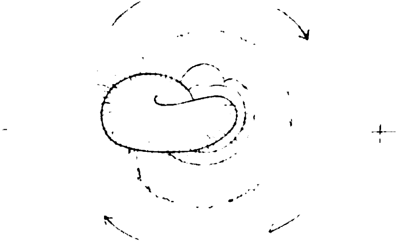

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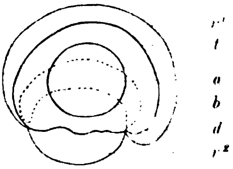

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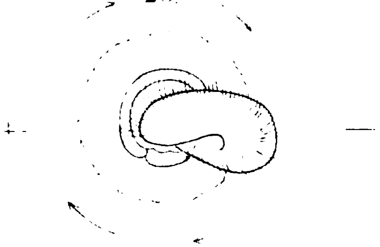

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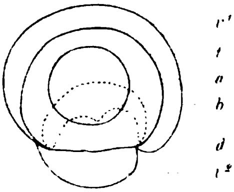

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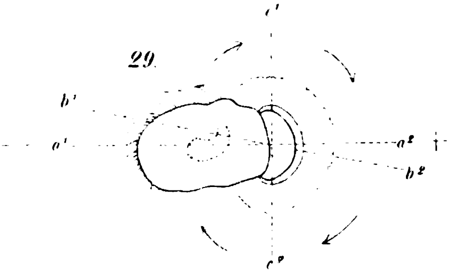

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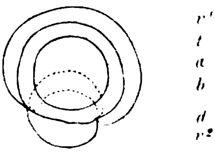

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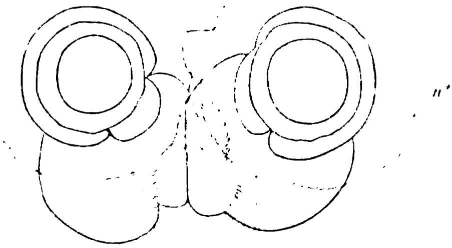

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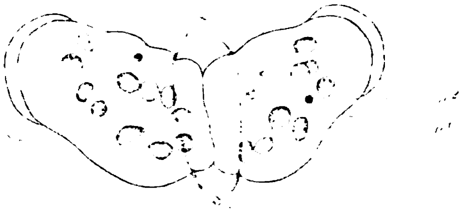

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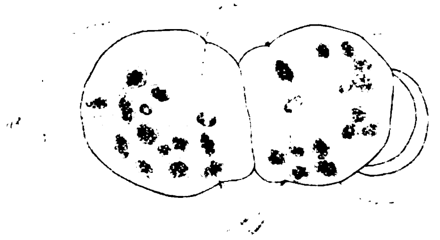

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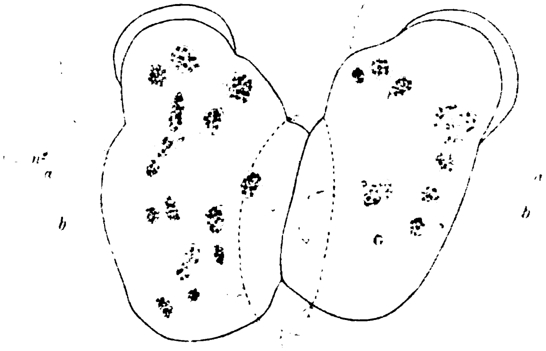

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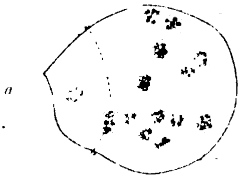

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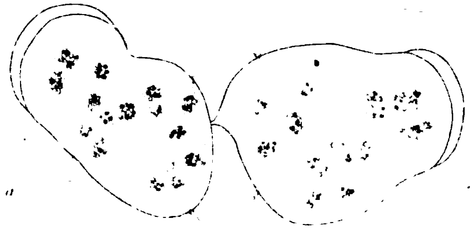

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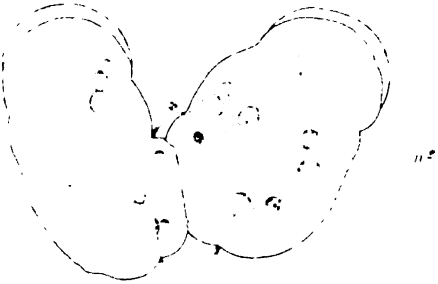

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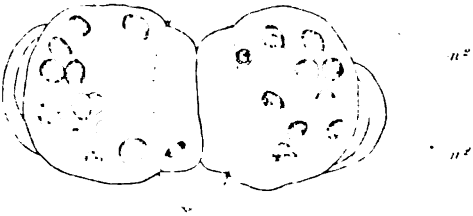

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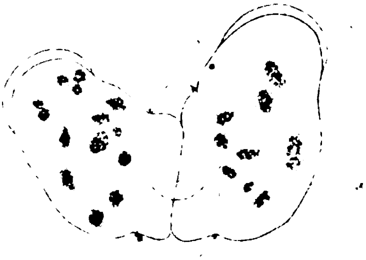

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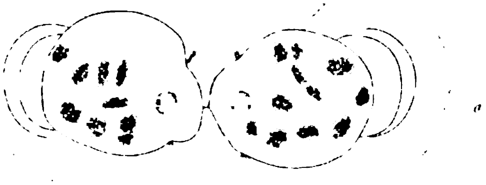

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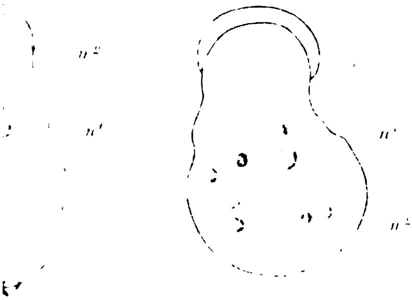

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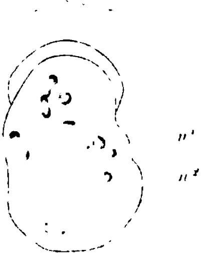

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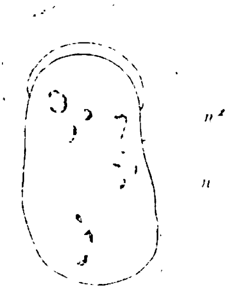

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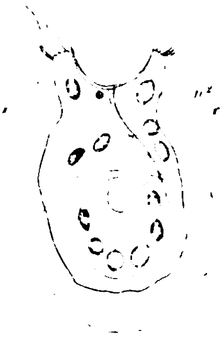

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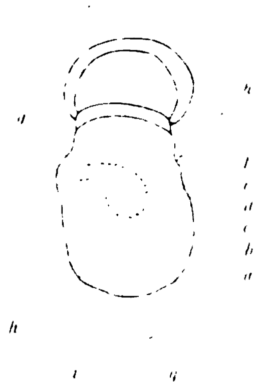

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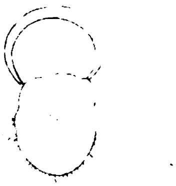

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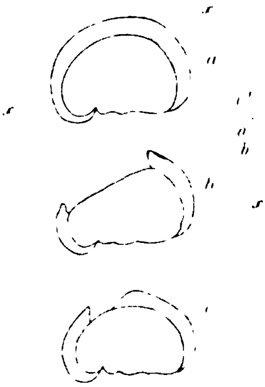

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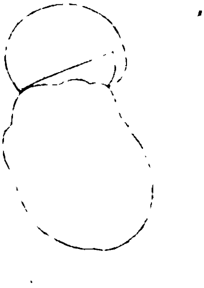

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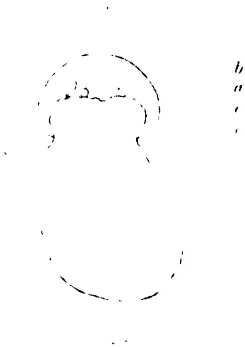

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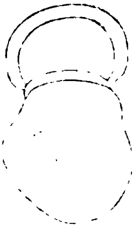

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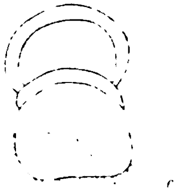

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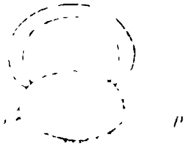

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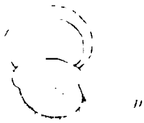

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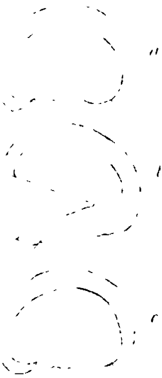

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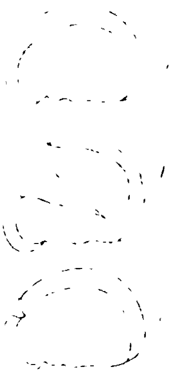

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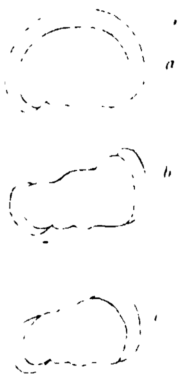

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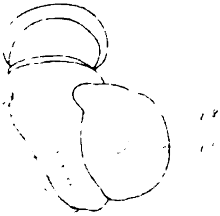

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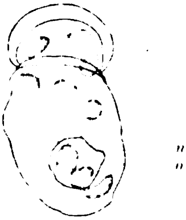

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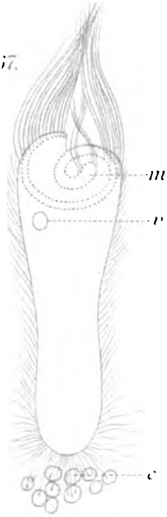

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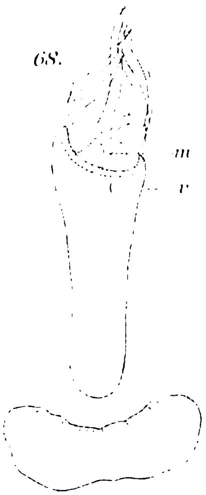

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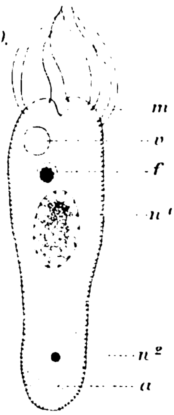

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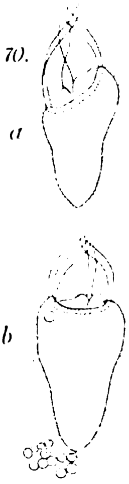

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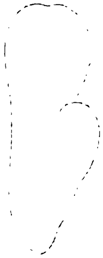

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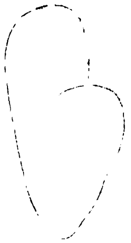

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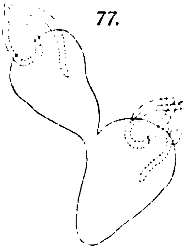

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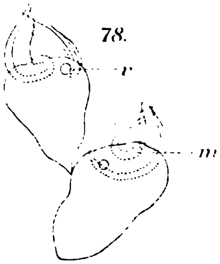

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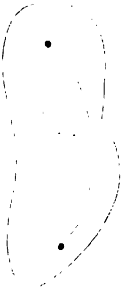

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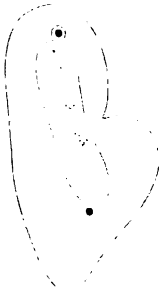

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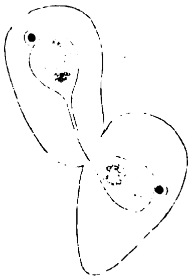

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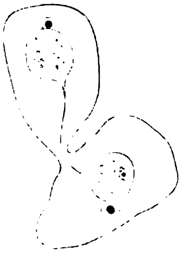

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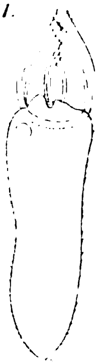

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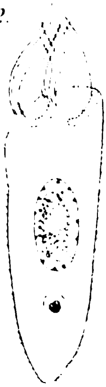

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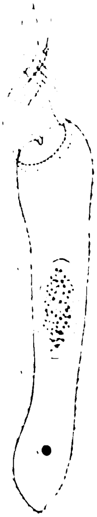

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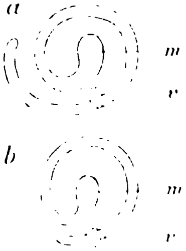

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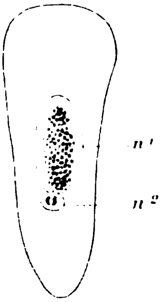

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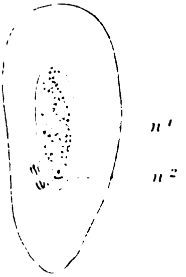

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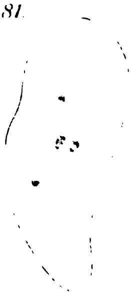

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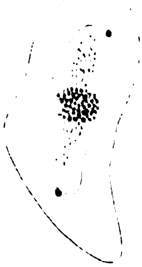

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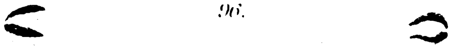

[Reprinted from BIOLOGICAL BULLETIN, Vol. V., No. 6, November, 1903.]

ABSORPTION OF THE HYDRANTH IN HYDROID POLYPS.

H. F. THACHER.

In 1900 there appeared a paper[1] by Professor Loeb on the "Transformation and Regeneration of Organs," the first part of which contained a discussion of the process of absorption in campanularia hydroids. His results were obtained from a study of the effects produced on the polyps by placing them in shallow dishes of sea water, so that they were in contact with the glass; under these conditions he found that they were gradually transformed and at length absorbed completely into the stem. To summarize briefly Loeb's account of this process, he states that there is noticeable first a contraction of the animal into the cup, followed by the fusion of the tentacles and later by the withdrawal of the whole polyp — now a shapeless mass of protoplasm — into the stem. This complete transformation he ascribes to contact, since it "is certain that contact with sea-water favors the formation of polyps with their more solid elements, while the contact with solid bodies favors the formation of the more fluid material of the stem or stolon." It seemed probable that a histological examination of these changes, in which the hydroid is represented as transforming and *creeping back into the stem,* might prove of interest, since they involved a complete transformation of well-differentiated structures. Therefore, at Professor Morgan's suggestion, I worked on this subject at Woods Holl during the summer of 1902. I was able to obtain a table first through the kindness of the director, and later was appointed to the Bryn Mawr table.

On examining the literature it will be found that there are frequent references to the absorption or disappearance of polyps. Loeb finds for *Margelis* and *Antennularia* that the polyps

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[1] *The American Journal of Physiology,* IV., 1900.

"disappear" when their condition of growth is disturbed — *i. e.*, the former being brought into contact with a solid, the latter being suspended horizontally so that its relation to gravity is changed. *Eudendrium*, according to some workers, sheds its hydranths when brought into the laboratory, but I have also often found absorption occurring under the same conditions, and *Eudendrium tenue*, a smaller and more delicate form than *Eudendrium racemosum*, responds in this way even more constantly. *Pennaria*[1] has recently been examined by Cerfontaine who finds that the day after the hydroids have been collected "ca matérialse trourait dans un mauvais état, les polypes qui persistaient étaient morts, les parties mollas s'étaient retireés dans la perisarque et les extremitées du cœnosarque réduit s'étaient cicatricées. Si l'ou conserve les branches, en maintenant une circulation d'eau de mer, ou les voit souvent reprendre de la vigueur. . . . Ou peut de cette façon déterminer expérimentalement une repetition de la regeneration spontanée. A la suite des troubles brusques produits dans les conditions d'être de ces organismes, par la récolte, le transport, le changement d'eau, le changement de température, de lumière, etc., ou determine rapidement la destruction des polypes ; mais bientôt, il semple se produire une acclimation rapide, et aussitôt une nouvelle régénération commence." *Tubularia* never absorbs its polyps but sheds them soon after being collected, and after a day or so if undisturbed, new polyps grow out from the old stalk, a new growth of stalk also taking place behind the head.

It seemed possible that the absorption of the heads of *Campanularia* might be analogous to that in these other forms, in which case it should occur even when not in contact with solids. To test this, I left the hydroids still growing on bits of wood, and placed them in the dishes, so that they were completely surrounded by water. Nevertheless the polyps began to absorb and by the end of twelve hours had almost entirely disappeared, while a few new ones were beginning to form from the old stalks. I also noticed on examining dishes of unused hydroids that had been standing over night, a large percentage of absorbing polyps.

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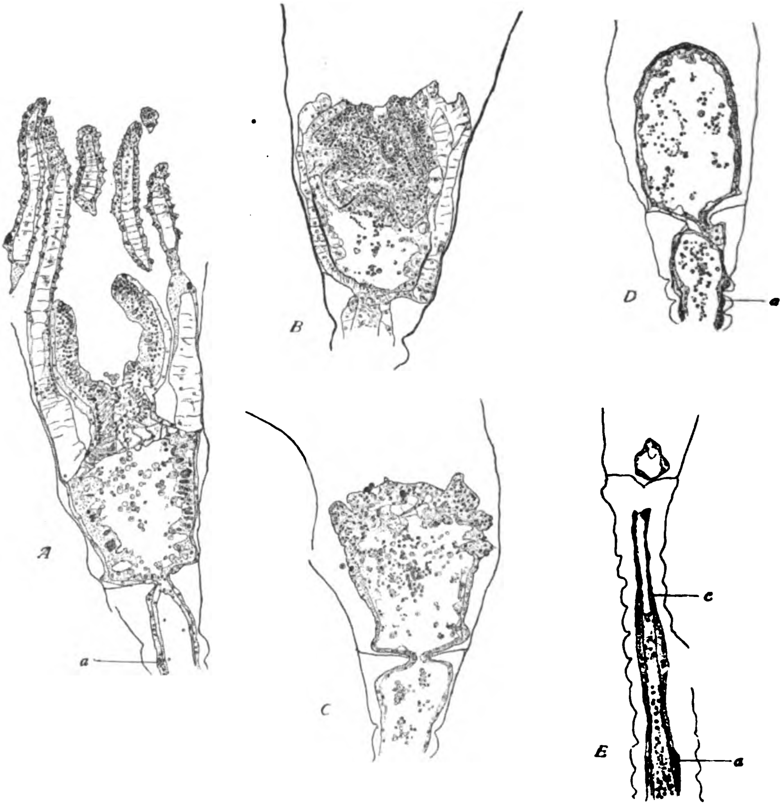
[1] "Recherches expérimentale sur la Régénération et l'Hétéromorphose chez astroides calycularis et Pennaria Carolinii," *Archives de Biologie*, XIX., 1902.

These results show that contact cannot in any case be considered the only factor to which the absorption of campanularian polyps is due, and that the process closely resembles that in other polyps in which under similar conditions we find either absorption or direct shedding of the hydranths with subsequent regeneration.

The material for study was obtained fresh each day, so that the animals should be in thoroughly good condition. Pieces of *Campanularia* were then cut and laid in watch crystals in contact with the glass in the way described by Loeb. The stages in the absorption of *Eudendrium* and *Pennaria*, which I used for comparison, being more difficult to obtain, were taken whether in contact or not, according to where they presented themselves. All the material was killed in cold corrosive acetic, and stained with Delafield's hæmatoxylin and congo red.

Within a few minutes after the removal of a piece or stalk, the cut end closes over, and the digestive current begins to flow slowly from one end of the hydroid to the other. It passes forward, and then is driven backward mainly by the contraction of the circular muscles of the polyps in the region just below the tentacles, but not involving a contraction of the whole animal; a slight pause occurs between each change in direction. The irregularity in the contraction of the polyps sometimes complicates the course of the current. At first the polyps remain expanded, and the only change noticeable is in the digestive fluid which becomes more and more laden with spherical granules of all sizes. The current is sometimes driven with such force that the contents break their way through a newly formed stolon or through the mouth of the polyp. The animal has up to this time been fully expanded except for the rhythmic contractions which decrease only the diameter of the body, but now it gradually contracts into its cup, and the body becomes shorter and broader, the latter change being largely due to the thickening of the ectoderm as can be seen even in the living animals. The tentacles undergo excessive contraction, becoming a crown of mere stubs, and then disappear altogether; their cells passing into the cavity of the polyp. At the same time, the hypostome absorbs.

These changes take some time and normally occupy at least two thirds of the time required for the complete disappearance of the polyp; sometimes the digestive current may, at this stage, distend the degenerating polyps and delay absorption for several hours. The usual time required is from six to twelve hours, but under the same conditions it may last from one to two days. The size of the structure left in the cup becomes slowly less and less, and at last the tiny ball of matter is drawn into the stem. I examined the living material carefully for signs of the breaking of the protoplasmic threads that stretch from the cœnosarc to the perisarc just below the cup, but I was unable in most cases to find any trace of it, until the last stage. At that time the strands break and the cœnosarc is drawn out in a fine thread. The protoplasm has been under a strain for the greater part of



the time, due to the growth of the stolon, but the protoplasm of the polyp cannot apparently be *drawn* through into the stem until it has reached a certain stage in its absorption.

The finer structure of normal *Campanularia* is as follows: The ectoderm cells which are flat on the body become cubical on the hypostome; there are no nettle cells except an occasional wandering one, until we come to the upper half of the tentacles. Below the cup lie masses of nettle-forming cells, somewhat irregular in their position, but never found in an quantity anterior to the first annulation. The endoderm is well differentiated on the hypostome into deeply-staining goblet cells and long spindle-shaped cells; in the walls of the body cavity there are large, clear endoderm cells and smaller granular gland cells. The tentacles contain a single row of endoderm cells. These are separated from those of the body cavity by a lamella at the base of the tentacle. Signs of change first arise in the endoderm of the body and the digestive current becomes filled with degenerating endoderm and gland cells, pinched-off portions of cytoplasm and loose nuclei. This process continues for some time without the appearance of any other change, except that as the endoderm becomes less, the lamella slowly contracts, becoming correspondingly thicker, and the ectoderm, having less surface to cover, changes from a thin layer to a much thicker one. The tentacles have also contracted to an abnormal extent, and at last by the breaking of the lamella across their base the endoderm cells round up and pass out into the body cavity. At this stage the tentacles are crowded together, and, the ectoderm being thrown into folds by the excessive contraction, frequently give, in surface view, the effect of being fused, as stated by Loeb. But by careful study the independence of the tentacles can be traced in spite of the closeness with which they are pressed together.

Soon after the endoderm has begun to pass out from the tentacles the lamella breaks near the tip and masses of nettle and ectoderm cells are poured into the cavity. The hypostome also degenerates, the ectoderm cells passing out rapidly into the digestive current and the lamella contracting after them. Soon the lamella of the hypostome breaks and disappears and the mass of ectoderm is also turned in. The polyp is now simply a shell of ectoderm and endoderm which are separated by the elastic lamella, which usually meets more or less completely at the oral end after the material of the tentacles and hypostome has been absorbed. At this time the lamella breaks in places and more cells from the ectoderm pass through. There is also a small amount of degeneration on the outside, and by these means the amount of ectoderm rapidly diminishes. Gradually the structure becomes smaller and smaller and finally the last fragment is drawn out of the cup. If there are many cells loose in the body cavity of the polyp at this time, they frequently break through the thin wall and pass out into the water.

The best guide by which to determine the amount of protoplasm drawn into the stem, was found to be the masses of nettle-forming cells before alluded to. The cells really drawn represent a very small fraction of the original number. The greater majority have been thrown into the digestive current, from which many are absorbed by the endoderm cells throughout the entire colony.

To compare the process in *Campanularia* with that in other hydroids, I examined both *Eudendrium* and *Pennaria* in which "absorption" also occurs and found the process again one of degeneration. From the time when the first degenerating masses are seen in the digestive current to the final drawing through of the small degenerated mass, the method is almost identical with that in *Campanularia*.

Recently there has appeared a paper by Gast and Godlewski, Jr., on the degeneration of the polyps of *Pennaria*[1] who have obtained results similar to my own.[2] It is interesting to note that their material was taken from polyps which had regenerated their heads in the laboratory, and then after two or three days had begun to absorb again — a different condition from that under which mine were obtained, yet the process is the same. Since these investigators have fully covered the ground for *Pennaria*[3] I shall not describe the changes in that form and indeed merely speak of two or three points in the degeneration of *Eudendrium* that differ from that in *Campanularia.* The degeneration of the endoderm is much more rapid, the cells breaking down more completely and filling the digestive cavity with fine protoplasmic granules. Since there is no lamella across the bases of the tentacles, the endoderm can also pass out from them more readily. The loss of ectoderm is here also accomplished by the passing in of cells through breaks in the lamella, the edges of which are apt to draw together again. The complete disappearance of the lamella does not occur until a very late stage. At the end the whole of the remaining structure is not always drawn through into the stalk, but an ill-defined mass of protoplasm is often left at the end.

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[1] "Ueber den Regulationsersheinungen bei Pennaria carolinii," *Archiv für Entwickelungsmechanick der Organismus*, XVI., 1903.

[2] See preliminary note, BIOL. BULL., IV., 2, 1903.

[3] Probably another species.

The constant position of the ectodermal gland cells near the beginning of the stalk throughout the degenerative changes show that there is no drawing of cells into the stem until the final stages.

The histological evidence thus supports my observations on the living animals, that in *Campanularia* we have to do with no transformation of the protoplasm due to contact, but with a degeneration of the polyp. Similar changes take place in other hydroids, and occur apparently when they are subjected to abnormal or harmful conditions.

I wish to express my thanks to Professor Morgan for his suggestions and kind supervision of my work.

The Regeneration of a Whole Foot from the Cut End of a Leg Containing Only the Tibia.

By

Margaret A. Reed.

With 3 figures in text.

Eingegangen am 3. August 1903.

The special problem, which I undertook to study at the suggestion of Professor MORGAN, was as follows. The fibula of the hind leg of a salamander, Spelerpes ruber, was removed and after the wound had healed the leg was cut squarely off though the distal end of the remaining bone, the tibia. The purpose of the experiment was to determine whether a whole or a part of a foot would be regenerated.

The experiment was carried out in the following way. The salamander was etherized and the fibula removed. This was done by first cutting the skin with small scissors and seperating the muscles from the bone, if possible without cutting the large blood vessels. Then, with the blunt end of a scapel, the bone was disarticulated and removed. After the bone was taken out, the cut edges of the skin were sewn together with silk thread and the animal was washed in distilled water. All the instruments used were sterilized by boiling water.

The salamanders were kept in glass dishes lined with wet filter paper, and covered so as to exclude the light. These dishes were kept in a cool place for the first few days in order to lessen the danger of gangrene.

After about seven days, when the wound had completely healed, the leg was cut off through the distal end of the tibia. It was difficult to be sure where the knee-joint lay and in a few cases, by mistake, the femur was cut, as sections subsequently showed. This made the individual unfit for the experiment, as the femur would then regenerate a new tibia as well as a new fibula.

The cut surface closed in a few days, and a new leg had begun to regenerate in from one to two weeks. At different stages in the regeneration, the leg was cut off near its base, and prepared for sections. It was put into corosive acetic (corosive sublimate, 95 cc + glacial acetic, 5 cc), hardened, decalcified in nitric acid (H_2O 98 cc + HNO_3, 2 cc; or 70 % alcohol, 98 cc + HNO_3, 2 cc), embedded in hard paraffine, sectioned, and stained on the slide with borax carmine. This stains the new cartilage cells darker than the surrounding tissue and enables one to trace the new bones, which are laid down in cartilage.

Four sets of experiments were carried out at different times during the winters of 1901/02 and 1902/03.

Tables I—IV.

I.

	Bone removed	Leg cut off	Reg. leg cut off	Result
1	Dec., 16, 1901	Dec., 23, 1901	Feb., 25, 1902	cut through femur
2	-	-	March, 4, 1902	femur injured
3	-	Jan., 3, 1902	March, 10, 1902	distal end of the tibia proliferating cells to form a whole foot
4	-	Jan., 6, 1902	Feb., 25, 1902	sections lost
5	-	-	March, 4, 1902	femur injured

II.

1	Feb., 7, 1902	Feb., 18, 1902	April, 19, 1902	distal end of the tibia proliferating cells to form a whole foot
2	Feb., 8, 1902	-	-	femur injured

III.

1	April, 7, 1902	April, 14, 1902	June, 3, 1902	cut through the femur
2	-	died		
3	-	April, 14, 1902	May, 28, 1902	femur injured
4	-	-	May, 26, 1902	-
5	-	-	-	-
6	-	-	May, 28, 1902	distal end of tibia proliferating cells to form a whole foot
7	-	-	May, 26, 1902	femur injured
8	-	died		
9	-	April, 14, 1902	May, 19, 1902	too young

IV.

	Bone removed	Leg cut off	Reg. leg cut off	Result
1	Oct., 27, 1902	Nov., 11, 1902	Dec., 22, 1902	small piece of fibula left
2	-	-	Dec., 17, 1902	femur injured
3	-	died		
4	-	Nov., 14, 1902	Jan., 27, 1903	tibia proliferating cells to form a whole foot
5	-	-	-	tibia proliferating cells to form new foot with 3 toes
6	-	-	Dec., 22, 1902	femur injured
7	-	-	-	-
8	-	-	Dec., 17, 1902	too young
9	-	-	Jan., 21, 1903	tibia proliferating cells to form a whole foot
10	-	-	Jan., 6, 1903	femur injured

These tables show five cases where the tibia formed at its distal end material for the fibula of the regenerated leg, and four of these five have regenerated a whole foot. In one experiment, a small splinter of the fibula was left. This splinter of the fibula completed itself and produced the fibula of the regenerated leg. In the other experiments either the femur was cut, in which case the femur became the source of the material for both the tibia and the fibula of the regenerated leg; or the femur was injured, in which case the it proliferated the cells to form the missing fibula.

Wendelstadt in his experiments on regeneration of the bone in the leg of salamander[1]), found that when the ulna or the radius is disarticulated and removed without injuring the humerus, the missing bone is not regenerated, while if a fragment of either bone is left, or the humerus injured, the missing bone is formed again. His results show that the remaining bone does not regenerate laterally to form the missing bone.

In my own experiment, I also have seen no evidence of such a lateral regeneration of an intact tibia, nor on theoretical grounds should there be any expectation that such would be the case. On the other hand where the tibia is cut across at any level, as in my experiment, the cells, which arise from its cut end, give rise not only to the missing end of the tibia, but to the distal end of the fibula of the regenerated leg, as well as to a whole foot.

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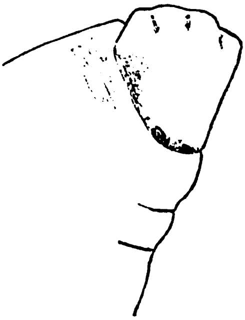
[1]) Über Knochenregeneration. Archiv f. mikr. Anatomie. 1901. LVII.

The one case, where the regenerated foot possessed only three toes, is probably due to the fact that the leg was removed before it was completely regenerated. Had it been permitted to grow further it would, no doubt, have formed five toes.

Fig. 1.



Since in the other four cases, the regeneration took place in about the same way, I will describe but one case that of No. 9 in set IV. The fibula was removed Oct., 27, 1902, and the leg cut off Nov., 14, 1902. Later, when the new foot showed the beginning of toes, Fig. 1, it was cut off and sectioned.

Fig. 2.

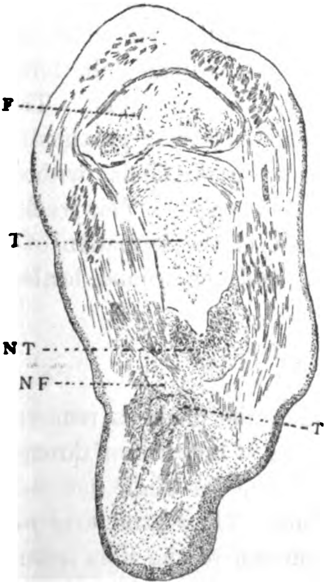
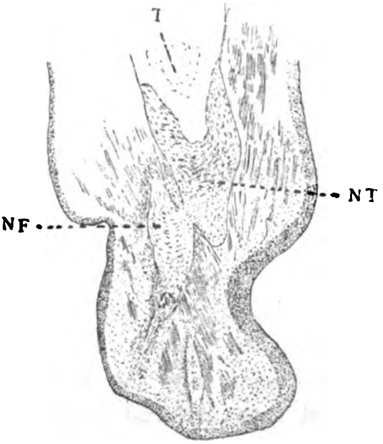


Fig. 3.



Tracing the sections through this regenerated leg, we find, Fig. 2, the old tibia articulated at its proximal end with the old femur (*F*); at the other end the tibia (*T*) shows a cap of new cartilage cells (*NT*). To the side of this are some of the cartilage cells (*NF*) which form the new fibula and also one tarsal bone (*t*), and one toe laid down in cartilage. Tracing on through the sections the femur becomes smaller and smaller until it disappeares altogether, the section of the old tibia becomes smaller, showing more and more cartilage cells of the new tibia and new fibula. Two more tarsal cartilages appear and the second toe. The sections then pass through the region where the new fibula is joined to the new tibia, Fig. 3. Other sections show the regenerated tibia and fibula connected with the tarsal cartilages, and the remaining three toes are completed.

We see that from the end of the old tibia, cartilages cells are given off forming the distal part of a new tibia, and also the tip of the fibula, but only that part of the fibula distal to the injury. The five toes are laid down in cartilage cells, and show that a complete foot is being formed from the material proliferated by the tibia.

Wendelstadt has shown that when a small distal piece of the ulna is left in the distal part of the fore arm, it will complete itself proximally extending upwards to the humerus, and form a normal arm again.

This, I think, is what would have happened in my experiment, also, had the leg been permitted to grow for a sufficient length of time; that is, the distal piece of the fibula would have grown proximally to form an articulation with the femur. In one case, where the new leg had grown for a longer time than usual, the fibula is well formed at its articulation with the tarsal cartilages, while the articulation with the femur is not completed, the cartilage cells forming only a slender tip instead of a large mass as for a joint. This seems to be a case where the fibula has completed itself proximally.

I also performed the experiment of removing the fibula, without later cutting the leg off through the tibia. At the end of four weeks, sections through this leg showed no evidence of the formation of a new fibula. The muscles were regenerated, and the wound healed, but no fibula was regenerated.

Summary.

When from the hind-leg of a salamander, the fibula is removed without injuring the remaining bones and the leg is later cut off through the tibia, the new part that regenerates will produce a whole foot with five toes, and also the distal end of the fibula. These structures are formed from material proliferated by the cut-end of the tibia alone.

Zusammenfassung.

Wenn von dem Hinterbein des Salamanders die Fibula entfernt worden ist, ohne die übrigen Knochen zu verletzen und der untere Theil des Beines später in der Tibia abgeschnitten ist, bringt der regenerirte Theil einen ganzen Fuß mit fünf Zehen sowie das distale Ende der Fibula hervor, das allein von Material gebildet wird, welches aus dem abgeschnittenen Ende der Tibia hervorgesprosst ist.

The Control of Heteromorphosis in Planaria maculata.

By

T. H. Morgan.

With 1 figure in text.

Eingegangen am 23. Oktober 1903.

Several years ago, in 1898, I found a case in which a short cross-piece of *Planaria maculata* regenerated a head on its anterior cut surface and another on its posterior cut surface. In 1900 I found that if the head of Planaria lugubris is cut off just back of the eyes a new head appears, as a rule, on the posterior cut surface of the head-piece. Later still (in 1902) I found that short cross-pieces through the region of the reproductive pore very often gave double-headed forms, — a head on each end. I also obtained a head on the posterior end of a head-piece of P. maculata. BARDEEN recorded (1902) finding a double-headed worm that had come from a cross-piece, and later (1903) he found that such pieces could be most frequently obtained from the pharyngeal region of the worm. He tried to connect this result with a supposed greater thickness of the nerve cords in this region.

The conditions that call forth these heteromorphic structures remained entirely unknown, and although I had tried several times to get an insight into the conditions in the cross-pieces, so that I could control their development, I did not succeed in doing so until the present summer of 1903.

I had observed that when the cross-pieces were moderately long (longer than broad) a heteromorphic head did not develop, but a tail always appeared at the posterior end. I also recalled that the hetermorphic heads had never been seen to appear when whole worms were cut into two long pieces through the same regions in which short pieces gave heteromorphic heads. It appeared to me therefore possible that the result was connected with the shortness of the cross-pieces. In order to test this view I carried out two series of experiments in one of which the pieces were longer than wide, and in the other much shorter than wide. In the former series no heteromorphic heads developed; in the latter a number of pieces gave double-headed forms, and it was very noticeable that this occured most frequently in the shortest pieces. Having determined this fact the question arose whether all the regions of the body have this capacity of producing hetermorphic heads. A number of worms were cut into short cross-pieces, and those from a given region of the body were kept together.

In the first experiment the heads of the planarians were cut off a short distance behind the eyes. Then the posterior ends of these head-pieces were cut off. In several cases the latter cross-pieces produced a head on each end, Fig. *A*. The head-pieces also produced, in several cases, a hetermorphic head.

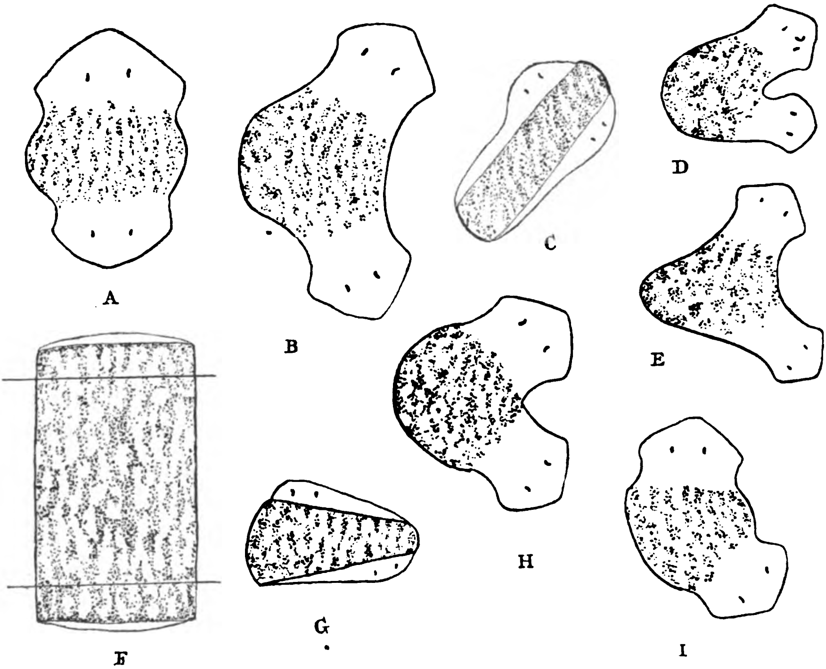
The region between the head-piece and the pharynx was also cut into cross-pieces, and, from several of these, double-headed pieces were obtained, Fig. *B*. The region of the body containing the pharynx was likewise cut into short cross-pieces, and these gave a similar result. Cross-pieces from the region behind the pharynx also gave some double-headed pieces, and here, once more, I seemed to obtain these double pieces more often than from the more anterior regions of the body; but whether this is only because shorter pieces are more easily obtained here, or because more of the very short pieces from this region survive the operation, remains an open question. Owing to the death of a large number of the very short cross-pieces it is difficult to determine whether one region is more prone to produce hetermorphic heads than another. The following experiments give some further results that bear on this point. The region between the head and the pharynx was cut up into very short cross-pieces. The greater number of these pieces died. Of the survivors, one piece was double-headed, five had a head and a tail, and one piece had a head on one end and the other end was closed. Of the short cross-pieces from the region of the pharynx of these same worms there was one two-headed piece, and the remaining fourteen had a head and a tail. Of the short pieces from the region behind the pharynx (to near the end of the tail), one had two heads, one had a head and a tail, and one had a head and a closed tail-end. These results are too meagre to show any greater tendency in one part than in another to produce double-headed forms.

At first sight it seemed hopeless to find an explanation that would account for the development of a hetermorphic head at the posterior end of these short cross-pieces, and also at the posterior end of the head-piece cut off just behind the eyes. The latter result appears to belong to the same category as the development of a hetermorphic head in the earthworm when the anterior end of the worm is cut off and kept alive by grafting on another worm. The regeneration of a heteromorphic tail in the earthworm from the anterior cut end of a tail-piece seems to call for a similar explanation, as does also the same process that takes place in the tail of the tadpole. In the first case the most obvious explanation is that the old head influences the new part in such a way that it produces another head instead of a tail, and in the latter cases the old tail influences the new part so that a new tail and not a head is produced. On the other hand in the double forms that develop from cross-pieces of the planarian it is not obvious at first how the same explanation could be applied, for these cross-pieces may come from any region of the body. If however it be assumed that the developing head on the anterior end of these cross-pieces exerts an influence on the new material forming at the posterior end, the result would be the same as in the other cases referred to above. This view might appear all the more plausible because the double heads appear only on short pieces in which the possibility of influencing the posterior material through the old part might be greater than in longer pieces. On this hypothesis, therefore, it might appear possible to bring under one point of view all the different cases of heteromorphosis. I attempted to put this view to the test of experiment with the results to be described in the next section.

The Position of the Heteromorphic Head in Oblique Pieces.

I had observed that the double-headed pieces not infrequently bulged out more on one side than on the other, as shown in Fig. *B*. In fact it appeared to me, at one time, that the production of the heteromorphic head might be connected with the obliquity of the posterior cut-surface, but I have convinced myself that no such necessary connection exists.

If the working hypothesis that I had formed were correct we should expect to find in oblique pieces the heteromorphic head, appearing opposite the other head, as in Fig. *C*, and since the anterior head lies, in oblique pieces, far over to one side, we should expect the heteromorphic head also to lie on one side and on the same side of the piece, as the anterior head, Fig. *C*. It should be pointed out that when a tail regenerates at the posterior cut-surface of an oblique piece, it appears also at one side, but at the more posterior side of the piece. Consequently the new heteromorphic head would not lie where the material develops fastest to make a tail, but at the opposite side of the posterior cut surface. This, in fact, proved to be the case.



A Double-headed worm from short cross-piece immediatly behind head. *B* From a cross-piece in anterior region of worm. *C* Diagram of oblique piece. *D* From an oblique piece like *C*. *E* From an oblique piece like *C*. *F* »Long piece«, whose ends were cut off at cross-lines, after 24 hours, to give short cross-pieces. *G* Diagram of a short piece with oblique anterior and posterior ends making an angle with each other. *H* From a piece cut off as shown in *G*. *I* From a piece cut off as shown in *G*.

Some of the different double-headed forms that developed from these oblique pieces are drawn in Fig's. *D*, *E*. At a stage like that represented by Fig. *D*. it would not be easy to determine whether the posterior head lies to one side of the posterior cut surface, as it appears to lie in the figure, or whether it occupies the entire cut surface, and bends forward as the worm crawls. In a few cases, however, in which the new head had only begun to develop it was not at all difficult to see that the heteromorphic head lay at one side of the cut surface, and on that side that is opposite the anterior head. This result seemed, therefore, to conform to the requirements of the theory.

There is, however, another fact which I had overlooked at first; namely, that the position of the heteromorphic head is what we might expect, a priori, even if the anterior head had no influence on its development. The heteromorphic head develops from the more anterior part of the new material on the posterior cut surface, and this is the position in which we should expect it to lie were it to develop independently of the anterior head; for it seems to be a rule in these planarians that a head develops from the most anterior part of the new material in which it appears. The result, while in conformity with the hypothesis, fails to establish the point of view.

It seemed to me possible to test the proposed hypothesis by means of a different sort of an oblique piece. If as shown in Fig. *G*, a narrow piece is cut out by two oblique cuts that make an angle with each other, the anterior head ought to appear to one side of the piece (the left in the diagram), and the posterior head ought to appear to the left (in the diagram) if it is formed under the influence of the anterior head, but to the right (as shown in the diagram) if its position is determined by the more anterior part of the new material on the posterior cut edge. This experiment ought to give a decisive answer to our problem. In practice, however, I found, that unless the oblique surfaces were very oblique, the new heads occupied so much of the cut surface that I could not determine to which side of the piece it lay. If on the other hand the pieces are cut off very obliquely then one side becomes necessarily so long that the piece gives rise to a head and a tail. I could not therefore solve the problem by this experiment, but in the future I shall hope to give it a further trial. In several cases I obtained two-headed worms from pieces of this kind, but, as shown in Figs. *H* and *I*, the new heads occupied apparently all of the cut-surface, or at least so much of each that one could not be sure that the head lay more to one side than to the other.

The Effect of Giving One End of a Short Cross-Piece a Start on the Other End.

A long cross-piece was cut out of the worm and its two cut ends allowed to heal over and to begin to regenerate. The anterior and the posterior ends of such a piece were then cut off after one, two, or three days, Fig. *F*. One end of each short piece would therefore have a start on the other. The short piece from the anterior end of the long piece would have the material on its anterior cut surface a day ahead of that on its posterior cut surface. Under these circumstances the head developing on the more advanced end ought, on the hypothesis, to produce a stronger influence on the material at the posterior end, than when the two ends were developing at the same rate. Conversely, the short piece cut from the posterior end of the longer piece would have the material on its posterior end more advanced than the material on the anterior end, and consequently we should expect there would be a smaller tendency for these pieces to produce double-headed worms, and possibly double tailed forms might develop.

In the first series, a long piece from the region between the head and the pharynx was cut out. After 24 hours the anterior and the posterior ends of these pieces were cut off, Fig. *F*, and all kept together. Of the four pieces that survived and regenerated two were double-headed. From the same worms long pieces containing the pharynx were also cut out, and after 24 hours the ends were cut off. Of the surviving pieces one out of seven had a double head, the rest had each a head and a tail. The long piece from the region behind the last was treated in the same way. Of the six surviving end-pieces each had a head and a tail.

Another similar series gave for the short pieces from the anterior region two double-headed pieces out of the four that survived; from the middle (pharynx) pieces two out of eight were double-headed. Of the pieces from the reproductive region there was one double-headed piece out of the four that survived. In this series the tail end was also preserved, and its anterior cut surface allowed to close and to begin to regenerate, when it was cut off as in the other experiments. Of these short cross-pieces two out of seven gave double heads.

With the exception of the last tail-pieces the short pieces from the anterior and posterior ends of the longer pieces were kept together. The results are, therefore, from one point of view inadequate to answer our question, since of the surviving pieces I could not tell which had come from the anterior and which from the posterior ends of the larger pieces. In the following experiments the anterior pieces were kept together and the posterior also by themselves. In the first series 48 hours elapsed before the end pieces were cut off, and in the second series 72 hours.

Of the small pieces from the anterior ends of the long pieces (from the anterior region), all of the four surviving pieces had a head and a tail, and of the pieces from the posterior ends all three had a head and a tail. Of the small pieces from the anterior end of the long pieces from the middle region all five had a head and a tail and from the posterior ends three had a head and a tail and one was two-headed. Of the small pieces from the anterior end of the long pieces from the posterior region all six had a head and a tail and this was true also for the posterior ends of the same pieces.

These results, far from showing an increase in the number of double-headed pieces from the anterior ends, show, if anything, the lack of heteromorphic heads in such pieces. That this was not due to any peculiarity in the worms that had been used was shown by the fact that after the ends had been cut off, the middle regions of the long pieces were cut up into very short cross-pieces, and of those that survived six out of thirteen had double heads; that is, nearly half of them were double-headed.

In the other experiment of the same sort, in which a longer interval elapsed after the first cut, each of the end pieces had a head and a tail. The pieces may have been too long, but the fact remains that fewer double-headed worms were obtained than when both ends started to regenerate at the same time. From this experiment, which should however be carried out on a larger scale, it does not appear that the start given to the anterior end results in a larger number of double forms, as we should expect on the hypothesis. In the light of this result it seems to me probable that this point of view is not correct.

Discussion and Conclusions.

It has seemed to me desirable to give a narrower meaning to the term heteromorphosis than that given to it by Loeb and by several recent writers. I apply it only to those cases in which the new part shows a reversed polarity with respect to the part from which it arises. The relatively few cases of axial heteromorphosis that are known have been obtained, with the exception of certain hydroids, when pieces near the ends of the animal have been cut off, and it may appear from this that there is some peculiarity connected with the ends that brings about this result. On the other hand this idea has failed to throw much light on the phenomenon, and is clearly inapplicable to the present case of Planaria. It is desirable, therefore, to look in some other direction for an answer to our problem.

In the Hydroids it has been shown in Tubularia, in Eudendrium, and perhaps in Antennularia antennina, that an external factor brings about the development of heteromorphic structures. In the case of Tubularia, at least, the action of the water on the posterior end appears to cause a reversal of the polarity. In Antennularia ramosa on the other hand it has been shown by STEVENS that the region of the stem from which the piece is taken is the most important factor in the result. Curiously enough the pieces from the distal end appear to have a strong tendency to produce roots at both ends, while pieces from the basal part of the stem generally produce stems at both ends. The problem is rather a special one here, for, it appears, that the so-called roots are, in reality, stolons that have the power to produce new stems.

The results that have the most fundamental bearing on this question of polarity are those of PEEBLES and of KING on Hydra. Pieces of hydra produce as a rule a head on one end and a foot on the other end. If, however, two short pieces are grafted together by their anterior ends one of the free ends will produce a heteromorphic head and the other a normal foot. The polarity of one piece has been reversed and a single and complete individual formed. The most important fact in this connection was determined by grafting pieces of different lengths together, when it was discovered that it is the larger piece that causes the reversal of the polarity of the smaller piece, so that the latter develops at its free end a heteromorphic head (or rather hypostome, mouth, and tentacles).

On first thought it may appear that this result in hydra is the same as that, which, on the hypothesis suggested above, was supposed to take place in the short pieces of planarians in which I assumed that there is also an influencee through the old part so that the polarity of the material at the posterior end is reversed. There is, however, a fundamental difference between the two cases. It is the polarity of the larger component of the united pieces of hydra that is supposed to change in its own direction the polarity of the smaller piece, while in the short cross-pieces of planarians the influence of the anterior head would have to be assumed to be of such a sort, that it causes the material at the posterior end to become polarized in the opposite direction. This consideration also goes to show that the hypothesis first suggested is probably incorrect.

Those who hold the view that regenerative phenomena can be accounted for on the assumption that there are reserve cells in the different region of the body that are potent to produce certain structures, heads and tails for instance, might attempt to account for heteromorphosis by the supposition that at the ends of the body only certain kinds of these cells exist, head-forming cells in the head region and tail-forming cells in the tail region. This way of accounting for the results has, I think, been found, unsatisfactory. In the first place, the new head and the new tail do not come from a single cell, but from a large number of cells that all combine to produce a single structure. Each cell must have a very wide range of possible regions that it may occupy in a new part. In the second place, it would clearly be absurd to attempt to apply this sort of an explanation to the double-headed pieces of planarians since heteromorphic heads may appear at any level. Furthermore since each level may become the anterior or the posterior end of a piece we must conclude that in longer pieces at every level a head or a tail may develop and the presumption is that a head or a tail may develop out of the same cells, but this is not essential to the present consideration. It is evident, I think, that in longer pieces the result depends on the relation of the new to the old part, and the question arises as to what this relation consists in. At present we can only refer it to the phenomenon that we call polarity. Here also we must look, I believe, for the solution of our present problem of the heteromorphic head. I offer, therefore, the following tentative hypothesis.

In very short pieces the two ends are so nearly alike, or what amounts to the same thing, the polarity of the piece is so slight, that it has no deciding influence on the kind of new part that develops. Under these circumstances we must assume that the new material that appears over a cut surface always produces a new head in Planaria maculata. At present we have little evidence from which to decide whether it is an internal or an external factor that determines, in the absence of polarity, that a head regenerates. In Planaria maculata it seems that the material throughout the body will produce a head unless the polarity of the piece decides that the new part shall become a tail. In the earthworm we shall have to assume that in the head-region there is a stronger tendency of the new material to make a head, even at the posterior end, if the piece is so short that its polarity ceases to be effective. In the tail-region, on the other hand, we must assume that there is a stronger tendency for the new material to make a tail, even at the anterior end, if the polarity of the piece is reduced. I suggest this as a tentative hypothesis and am not unaware that the assumption can not be conclusively established at present. As a working hypothesis however the ideas here suggested may, I hope, lead to further experiments.

It has been supposed by BARDEEN that the heteromorphic head in planarians is due to the influence of the nervous system that is exposed at the posterior cut end, and since he obtained these heads more frequently, in the region of the pharynx, he examined the nerve cord in that part and thought on the whole that it was somewhat thicker there. That he may have found this condition in one or more cases (he does not state how many individuals seemed to show this) is not surprising to any one familiar with the action of killing fluids on planarians, which cause them to contract violently, and the extent of the contraction is often different in different regions. I have taken the trouble to cut into serial sections three individuals of *Planaria maculata* and can state that no such thickening occurs, but the nerve-cord gradually tapers from its anterior to its posterior end. The inadequacy of BARDEEN's assumption is further apparent, since I have been able to obtain these heteromorphic heads at all levels in the worm. In the light of these facts I do not think BARDEEN's suggestion can be given very serious consideration.

This notion of BARDEEN's is in line with a more general suggestion that he has made as to the supposed influence of the nervous system in the regeneration of a new head. It would not be profitable to discuss this point at length, since the only observations, or rather deductions, on which it rests can now be shown to be erroneous. He states that longitudinal pieces cut far out at the side of *Planaria maculata*, so that the longitudinal nerve-cords are not cut, are incapable of forming a new head, or at least he did not obtain any such results from them. From this faillure to obtain heads he concludes that the result is due to the absence of nerve-cords in the pieces. At my suggestion Miss N. M. STEVENS cut off side pieces from *Planaria lugubris.* That these pieces did not contain any part of the nerve cords was demonstated by cutting sections of the worm from which each piece had been removed. In *Planaria lugubris* this operation is very easy, since the nerve cords do not lie so far out at the sides as they do in *Planaria maculata.* The side pieces always produced heads!

In order that it might not be said that this experiment was made on a different species from that which BARDEEN employed and therefore did not apply, I have carried out the same operation on large individuals of *Planaria maculata*, and in all cases in which the pieces were large enough I found that new heads developed. The original worms were in each case also sectioned, and these showed that the nerve-cord had not been cut. Therefore until BARDEEN brings forward better evidence in favour of his conjectures than he has as yet produced, we must look, I believe, in other directions for an explanation of the regeneration of heteromorphic heads[1]).

Finally I should like to point out that narrow pieces from the side without any nerve cord in them, often produce a head at the cut edge using up, in its formation, all of the material at the side. This result, which I first observed in 1898, and described quite fully, has interested me for some time, but its explanation did not occur to me until I had formed an opinion as to the cause of the origin of the heteromorphic heads. The explanation is I think the same in both cases; namely, it is due to the absence of sufficient polarity in the short pieces to determine that the head shall be limited to a part only of the new material. All of the new material, if the piece is not too long, is used up in the formation of the new head. If the piece is long two heads are often found side by side in the new material and no other structures are produced. Here also we find, I think, fairly good evidence pointing to the conclusion that in the absence of polarity the new material produces a head.

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[1]) The question as to whether side pieces without any of the two main nerve-cords in them can produce a head is a different one from that as to whether, if a piece of the nerve-cord is present, it may not have some influence in locating the new nerve-cord. BARDEEN has not, I think, sufficiently kept this distinction in mind. Even in narrow lateral pieces some of the lateral nerve-paths are present, but whether they play any part in locating the brain in the new head remains to be determined.

Summary.

1) The regeneration of a head on the posterior end of a cross-piece of *Planaria maculata* is due to the shortness of the piece; the shorter the pieces the larger the proportion of double-headed worms (Fig. *A*) that are obtained. Long pieces do not produce heteromorphic heads.

2) Short oblique pieces (Fig. *C*) also often produce heteromorphic heads. The posterior head lies at the same (lateral) side of the piece as does the anterior head (Fig. *C*, *D*, *E*).

3) If a long cross-piece (Fig. *F*) is cut out and its ends allowed to begin to regenerate, and if then its anterior end is cut off, there is no greater proportion of double-headed worms obtained than when short cross-pieces have both ends regenerating at the same rate. This result indicates that the development of the heteromorphic head is not due to the influence of the anterior head.

4) Our analysis of the conditions that lead to the devolopment of the heteromorphic heads in short cross-pieces of *Planaria maculata* leads to the conclusion that there is always a stronger tendency in the material that develops over a cut surface to produce a head than to produce a tail, and that a head will appear unless the polarity of the piece is sufficiently strong to overcome this tendency, and cause a tail to regenerate. Long pieces therefore produce a tail at their posterior ends, and only very short pieces, in which the polarity is reduced, a heteromorphic head.

5) A similar explanation is extended to other cases of axial heteromorphosis. In some of these, as in the earthworm, it is assumed that in the anterior region the new material is more strongly predisposed to produce a head, and in the tail region a tail. When the polarity is reduced in these regions the heteromorphic structure appears.

6) Pieces from the side of *Planaria maculata* and *Planaria lugubris* that do not include any part of the main nerve cord regenerate a head. The lateral position of the heads in these pieces is probably also connected in part with the lack of strong polarity in the pieces.

Zusammenfassung.

1) Die Regeneration eines Hauptes am hinteren Ende eines durch Querschnitte erhaltenen Stücks von Planaria maculata ist von der Kürze des Stücks abhängig; je kürzer das Stück, in desto größerem Verhältnis treten die erhaltenen zweiköpfigen Würmer auf (Fig. *A*). Lange Stücke produciren keine heteromorphischen Häupter.

2) Kurze, schräg geschnittene Stücke (Fig. *C*) erzeugen gleichfalls oft heteromorphische Häupter. Das hintere Haupt liegt auf derselben (lateralen) Seite wie das vordere (Fig. *C*, *D*, *E*).

3) Wird ein langes Stück durch Querschnitte herausgeschnitten und der Regenerationsanfang an seinen Enden abgewartet, darauf sein vorderes Ende abgeschnitten, so treten doppelköpfige Würmer nicht in stärkerem Verhältnis auf, als wenn bei kurzen Querschnittsstücken die Regeneration beider Enden gleich weit fortgeschritten ist. Dieses Ergebnis zeigt, dass die Entwickelung des heteromorphischen Hauptes nicht auf einem Einfluss des vorderen Hauptes beruht.

4) Unsere Analyse der Bedingungen für die Entwickelung heteromorphischer Häupter an kurzen Querstücken von Planaria maculata führt zu dem Schluss, dass das an einer Schnittfläche sich entwickelnde Material stets eine stärkere Tendenz hat, einen Kopf als einen Schwanz hervorzubringen und dass ein Haupt erscheint, außer wenn die Polarität des Stücks stark genug ist, jene Tendenz zu überwinden und die Regeneration eines Schwanzendes zu veranlassen. Lange Stücke bringen daher einen Schwanz an ihrem Hinterende hervor, und nur ganz kurze Stücke, in denen die Polarität reducirt ist, erzeugen ein heteromorphisches Haupt.

5) Eine ähnliche Erklärung erstreckt sich auf andere Fälle axialer Heteromorphosis. In einigen von ihnen, wie beim Regenwurm, wird angenommen, dass das Material am Vorderende stärker zur Erzeugung eines Hauptes prädisponirt ist, in der Schwanzregion entsprechend zu der eines Schwanzes. Wird die Polarität reducirt, so erscheint in diesen Bezirken das heteromorphische Haupt.

6) Aus den Seitenbezirken von Planaria maculata und Planaria lugubris entnommene Stücke, welche keinen Theil des Hauptnervenstranges enthalten, regeneriren ein Haupt. Die seitliche Stellung der Häupter bei diesen Stücken hängt wahrscheinlich mit dem Mangel starker Polarität bei diesen Stücken zusammen.

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NOTES ON REGENERATION.

T. H. MORGAN.

During the past summer I made at Woods Holl a number of observations and experiments on the regeneration of several animal forms. The results are here brought together, although they have little more in common than that they all deal with problems of regeneration.

THE LIMITATION OF THE REGENERATIVE POWER OF DENDROCŒLUM LACTEUM.

The fresh-water planarians show such remarkable powers of regeneration that it is surprising to find in one of them, *Dendrocœlum lacteum*, that this power is much reduced. The question at once arises whether we can discover anything peculiar in the relation of this planarian to its surroundings, or in its internal structure that will give a clue to its exceptional behavior.

There is nothing in its habitat to suggest that it has lost, or has never acquired to the same degree, the power of regeneration possessed by other planarians. In the pond at Falmouth where I collected this species there were also present, sticking to the under surfaces of the same stones, both *Planaria maculata* and *Phagocata gracilis*. If *Dendrocœlum* is not as subject to injury as are the other two species, and if, therefore, it does not need the same regenerative power, it is remarkable that *Dendrocœlum* should be so uncommon in comparison with the other two forms. If it is subject to greater injury, then it has not acquired the power to meet the situation as have the other species. Considerations of this kind do not have, I believe, any real bearing on the question of whether an organism has or has not acquired the power to regenerate, although some biologists lay great stress on this sort of speculation. The limitations in the power of regeneration of *Dendrocœlum* are peculiar. Lillie found that when only the anterior end of the worm is cut off a new anterior end is regenerated. This power to produce a new head was found to extend back to about one-third of the length of the worm, *i. e.*, to a region just in front of the pharynx. Behind this level the posterior piece fails to regenerate a head at its anterior end.

On the other hand, the anterior pieces regenerate a new posterior end from any level, with the possible exception of the immediate region of the head itself; but the latter point has not yet been sufficiently examined in this species. It appears a remarkable fact that this planarian should have such extensive powers of regenerating posteriorly, and such limited powers of regenerating anteriorly, especially since, as far as we know, the same cells produce either a head or a tail according to which end is exposed; but this has not been definitely determined, and would be almost impossible to determine with absolute certainty. Eugen Schultz has also studied the regeneration of *Dendrocœlum lacteum* of Europe[1] and finds that posterior pieces do sometimes regenerate a head, although the regeneration is very slow, and it may appear that Lillie did not keep his pieces a sufficiently long time for the regeneration to take place. He states, in fact, that most of the posterior pieces died after five or six days. Schultz believes that these posterior pieces have potentially the power to regenerate, but that sometimes the piece closes in such a way that the formation of new tissue is prevented, as I have found to occur occasionally in *Bipalium*. Lillie, on the other hand, tries to account for the lack of power of posterior pieces to form a head by means of the following hypothesis. He suggests that the regeneration from the posterior cut surface at all levels is due, in some unexplained way, to the presence "of the brain and anterior part of the nervous system in the anterior piece." Conversely the absence of these structures in posterior pieces is supposed to account for the lack of regeneration from the anterior cut surface. A simple experiment would have shown the untenability of this point of view. If the head end is cut off just in front of the pharynx so that the brain and the anterior part of the nervous system are removed, and then the tail end of the middle piece is also removed, it will be found that the middle piece without regenerating a new head will still regenerate a new tail. This shows conclusively that Lillie's supposition in regard to posterior regeneration is erroneous. The remainder of his argument, which rests on this assumption, also falls, I believe, in the light of this fact.

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[1] It has been assumed that the European *Dendrocœlum lacteum* and the American form or forms are identical, but I think this question will bear further examination.

The great mortality that Lillie observed in the posterior pieces is due largely, at least in my experiments in which the same thing was observed, to the temperature being too high, or possibly to exposure to light. If the pieces are kept cooler (by surrounding the dishes by the cool, running salt water of the laboratory) the mortality is much reduced, and instead of dying after six days, as in Lillie's experiment, I have kept short posterior pieces for several weeks. It is only by keeping such pieces for a long time that one can fairly test their powers of regeneration.

Schultz states that he cut *Dendrocœlum* in two either between the pharynx and the reproductive region or else in front of the pharynx. In the former case he found that the posterior pieces regenerated an anterior end very slowly, and he found it more profitable in studying the regeneration of the head to use those posterior pieces that had been cut off in front of the pharynx. He found that the regeneration of the anterior end often failed to take place, and he attributes this to fusion of the sides of the cut surfaces, as I had found to occur not infrequently in *Bipalium*. Whether this is the whole of the question remains to be seen. In a marine polyclad, *Leptoplana*, Schultz found that posterior pieces, no matter at what level they have been removed, fail to regenerate an anterior end, even when only a small piece of the head is cut off. Yet regeneration from a posterior cut surface takes place at all levels. Schultz attributes the lack of regeneration at the anterior end either to the closing over of the "growing point" by the coming together of the old tissue from the sides, or to the muscles from the sides uniting and thus preventing further growth. Both factors he thinks may enter into the result. This point could be tested, I think, by making the cuts so that there is left a pointed anterior end, when regeneration should occur, if Schultz's view is correct. From an experiment of this sort that I have carried out on *Dendrocœlum* I think it probable that in *Leptoplana* also no better regeneration would occur, even at a pointed end,[1] and if this proves to be the case Schultz's explanation is insufficient.[2]

In my experiments I first examined whether the form of the cut surface at the anterior end had anything to do with the lack of regeneration, for it was possible here, as in the case of *Bipalium*, that the cross-cut surface closed in such a way that subsequent regeneration was prevented. By changing the form of the cut surface this difficulty should be eliminated. Posterior pieces were cut off through the region of the pharynx and also behind the pharynx. The anterior ends of some of these pieces were very oblique; others were pointed in the middle, *i. e.*, they were cut off by two oblique cuts meeting in the middle line. In the latter case especially it is impossible that the muscles from the sides could close the anterior cut surface.[3] These pieces were kept alive for two or three weeks, and although it could be seen that there was a little new tissue at the anterior cut surface, yet no further regeneration occurred after the first ten days or thereabouts, and there is no indication that regeneration would have gone any further if the pieces had been kept alive for a greater length of time.

Sections of these pieces were made. The results will be given below.

In two other series each worm was cut into three pieces. The head pieces extended to the middle of the region in front of the pharynx. These pieces should be capable of regenerating at the posterior end. The middle pieces included the next portion of the worm, and extended to the region of the reproductive pore. These pieces should be capable of regenerating a head at their anterior ends and a tail at the posterior ends. The third pieces were the tail pieces and included the rest of the worm. These pieces should be incapable of regenerating a head at the anterior end. The pieces were preserved at intervals of 1, 2, 3, 4, 5, 6 days, killed, embedded, stained and examined with immersion lenses.

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[1] Loeb says that *Thysanozoon* regenerates a new head, but he did not determine whether a new brain is formed. Monti also obtained regeneration in this form *and also in Leptoplana*, except when cut far posteriorly. Lang also records regeneration in marine polyclads.

[2] Schultz states in the opening of his paper that I carried out my experiments without making sections of the planarians, and he intimates that had I done so I would not have reached certain conclusions in regard to the growth of the new part. How Schultz obtained this information it would be interesting to know. Probably he based his generalization on the absence in my earlier papers of reference to histological details with which I was not then especially concerned. As a matter of fact I had made and studied many sections. My students also were at work on the minute anatomy, and one of them published a complete account of the histological changes taking place during regeneration before Schultz's paper appeared.

[3] Whether union of the dorsal and ventral muscles might close these pieces I have not here considered.

A study of the sections shows that the changes taking place at the anterior end of the tail-pieces appear to be similar in all respects to those that occur at anterior or posterior surfaces at which regeneration of the missing part takes place. There is nothing in the sections to show why the regeneration should continue in the one case and not in the other, and it is difficult to believe from the evidence of the sections that anterior regeneration from the tail-pieces would not in time be accomplished, yet after three weeks there was no sign of further regeneration and I am forced to conclude with Lillie that in the form of *Dendrocœlum* found at Falmouth regeneration does not, ordinarily at least, occur behind the level of the pharynx. Sections through tail-pieces, cut off behind the pharynx and kept for nearly three weeks, show that the formation of new tissue has not gone much beyond that of the first six days, and that a new head has not been produced. Sections of the oblique, and of the pointed tail-pieces give exactly the same results.

Several writers seem inclined to account for the lack of regeneration in certain planarians, and especially from the posterior region of the body, as due to the absence or small size of the nerve cords in these regions. With this view I do not agree. Lillie has used *Dendrocœlum* as a case in point. Sections of this worm show, however, that the cords in the more posterior regions are as well developed, judging from their size, as they are in *Planaria maculata*.

REGENERATION IN PYCNOGONIDS.

In 1895 Loeb published some observations that he had made on the regeneration of one of the Pycnogonida, *Phoxichilidium* *maxillare.* He cut the animal in two between the second and third pairs of legs, and found in two cases that after a time a new part suddenly appeared, presumably after a moult. This new part that regenerated at the posterior end of the anterior piece Loeb speaks of as a body, and points out that this is the first case observed in the arthropods in which new body segments have been seen to regenerate. I have repeated this experiment during two summers, for it did not appear to me beyond dispute that the new part that had been observed was necessarily a body, since no satisfactory evidence that it was such is furnished by Loeb's paper. Although sections of the new part were, apparently, made, no posterior opening of the digestive tract was found, no ganglia are described as being present in the new body, nor do new legs appear to have been present at the sides as we should expect if this new part were really a body.

My first experiments were made in 1901 and, although a number of pycnogonids were kept for two months or longer, none of them regenerated at the posterior end. Since I had used large individuals it seemed not improbable that the lack of regeneration might have been connected with the maturity of the individuals. During the past summer I have repeated the experiment on a large scale, both with large and with small individuals; but although many of the pieces were kept for nearly two months no regeneration took place, with the possible exception of two instances that will be described.

In a number of cases the individuals were cut in two between the third and fourth pairs of legs, *i. e.*, nearer the posterior end than Loeb had cut them, for, from analogy with other cases, it seemed more probable that if the body could regenerate at all it would be more likely to do so the nearer the cut was made to the posterior end. Other individuals were cut in two between the second and third pairs of legs. In only one case did regeneration appear to take place, as shown in Fig. 1. Here the bases of the fourth pairs of legs bulge out as though they had been formed anew, and it seems possible that the rudimentary abdomen is also new, although it is also possible that a part at least of this structure had been left unintentionally when the cut was made. Sections show that the digestive tract opens at the end of the abdomen. There is no trace of further regeneration within the stumps of the legs. At most, the bases of the legs and the abdomen, or part of the latter, have regenerated.

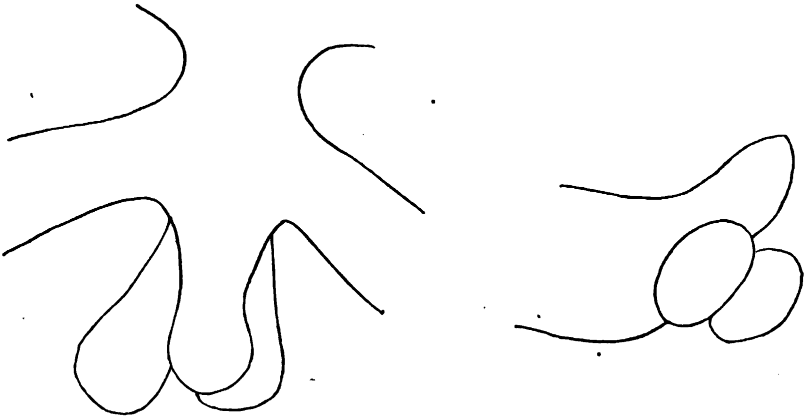

Fig. 1.

The second case is shown in Fig. 2, in which there is only a bulging out of the end of the body. The cut had been made in this case between the second and third pairs of legs. Sections of this individual do not show any indications of the development of legs or of a rudimentary abdomen in the new tissue of the bulging portion, and there is nothing to indicate that the development would ever have gone any further. The digestive tract ends blindly and is not connected with the ectoderm.

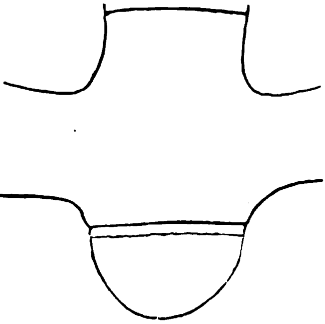

Fig. 2.

In looking over a large number of individuals I found a few cases in which a leg on one side was much smaller than its opposite, and from this it seems probable that the original leg had been lost at the breaking joint at the base, and a new one had begun to regenerate. Moreover, I found one case in which the new leg was clearly a new structure, Fig. 3. The different segments had not yet been formed in their adult proportions and the leg could not have been functional as yet. There is some resemblance between this leg and the newly regenerated part from the posterior end of the body that Loeb saw and figured. In fact this idea seems to have suggested itself to Loeb for he writes: "Das Vorhandensein eines ueberzähligen Segmentes könnte vermuthen lassen, dass das neugebildete Stück vielleicht im Laufe der Zeit sich zu einer Extremität entwickelt haben würde, dass es sich also um die Bildung eines Beines an Stelle des abgeschnittenen Rumpfstückes gehandelt habe, ein Fall, den ich als Heteromorphose bezeichnete. Allein Hoek führt an, dass bei Ammotheen das Abdomen nicht selten Spuren einer Segmentation zeigt." Thus in order to explain away the presence of too many segments in the new part Loeb has recourse to a condition found in another species — a mode of explanation that will scarcely recommend itself.

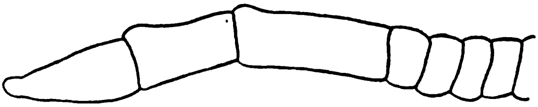

FIG. 3.

A somewhat fuller analysis of these two cases of Loeb's may not be unprofitable. If the new part is really a body, *i. e.*, thorax and abdomen, we should expect to find the digestive tract opening at the posterior end, but this does not appear to have been the case, for, Loeb says: "Der Darm setzte sich in den vorderen Theil des regenerirten Stückes fort. Im Uebrigen aber waren die Gewebe wenig differenzirt." It is to be remembered that the digestive tract also continues out into the legs in the pycnogonids as a blind sac. In the second place, while the three segments of his first example might be interpreted as representing the two remaining thoracic segments, and the rudimentary abdomen, yet in the other case five or six segments appear in the new part. It is this that led Loeb to suggest that the new part might represent a leg; but he withdraws this interpretation at once as seen above. There is certainly no striking resemblance between the new part figured by Loeb and the abdomen of *Ammothea.* Finally, if the new part is a new thorax where are the legs?

In the light of these considerations we must wait until some one, favorably situated, has an opportunity to work over the subject with ample materials. Meanwhile it seems to me that so far as the evidence goes it is rather in favor of the view that the regeneration described by Loeb is a new leg and not a part that replaces the lost segments of the thorax and abdomen.

The Lack of Regeneration of the Pigment Spot in the Fin of Fundulus.

If a gold fish having a black band at the end of its tail be selected, and the end of the tail be cut off proximal to the band, a new band like the one removed reappears in the regenerated tail. The presence of black pigment at the cut surface from which the new part regenerates is clearly not necessary for the development of pigment in the new part. This result is all the more curious since the occurrence of the pigment band is only an individual peculiarity. It seemed desirable to try the same experiment in a species in which a characteristic spot or a ring was present. The dorsal fin of the male of *Fundulus majalis* has a black spot in its posterior part, Fig. 4. The spot is not present in the female, and it appears, therefore, that this color marking belongs to the category of secondary sexual characters.

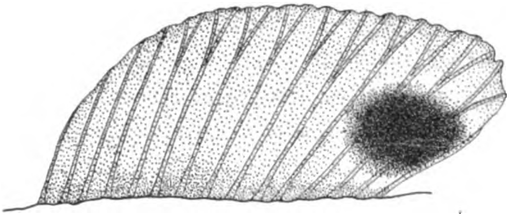

FIG. 4.

The posterior part of the fin was cut off by an oblique cut; the part removed containing all of the black spot. The lost part was slowly replaced, and in the course of two months the fin was completed, but the pigment spot did not come back, and there was no evidence that it would have done so if the fish had been kept longer. Since the operation had been carried out during the height of the breeding season, it seemed possible that the spot might normally fade out later, but other fish, examined in September, showed the spot still present.

The results on *Fundulus* appear to be different from those on the gold fish, and it is not apparent why this difference should exist. The result does not seem to be connected in any way with the fact that the spot in *Fundulus* is a secondary sexual organ. The most plausible explanation that suggests itself is that in the tail of a gold fish that has a black tip there are cells throughout the tail that can develop pigment should they get into the terminal portions of the tail, while no such cells are present in *Fundulus*, or if present they fail to produce pigment in the new part. It may be that in *Fundulus* all the cells capable of producing pigment have been already carried into the pigment-spot itself, and hence when this spot is removed no cells capable of developing this pigment are present in the remaining part. Further work will be necessary to determine whether these suggestions have any value.

THE METHOD OF CLOSURE OF THE CUT ENDS OF TUBULARIA.

The peculiar method of closure of the cut ends of *Tubularia* has attracted attention since it appears to be different from the closing observed in other forms. I have already discussed at some length this process[2] and shall not repeat here what has been already said, but since I have observed during the past summer certain processes that seem to throw some light on this question I shall briefly refer to them in this connection. Stevens has figured the closed end of a piece that had been cut through the hydranth-forming region at the time when the primoidium of the new hydranth had just been laid down, and when the red pigment lines, that indicate the appearance of the new hydranth, were present. Over the closed end the red lines radiate to the center of the bounding membrane. It seemed to me that a further examination of pieces that closed in this way might throw some light on the process in general. Pieces were cut off and

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[1] Morgan, *Roux's Archiv*, XIV., 1902.

[2] "Regeneration," 1901, p. 69.

kept about twenty-four hours when the primoidia of the new tentacles had begun to appear. At this time I cut the ends squarely off, the plane of section lying across the middle of the new proximal tentacles. To my surprise the cut ends now closed in a very different way from that of ordinary cross-cut pieces. The whole wall contracted from the perisarc and the cut edges were brought together almost at once, and subsequently fused, often showing the radiating lines described by Stevens over the new end. It was perfectly clear that the result was due to a contraction of the cœnosarc, and the difference between this process and that shown by ordinary pieces appears to be due entirely to the fact that at the time when the tentacle primoidia are laid down, the cœnosarc has become free from the outer wall, or perisarc.

From this result it seems to me to follow with great probability that in ordinary pieces the closure of a cut end is also due to a process of contraction of the cœnosarc, but ordinarily the wall of the cœnosarc is so closely stuck to the inner surface of the perisarc that it is not free to pull away as a whole, and there is a consequent drag that holds back the contracting wall, and a consequent modification of the method of closure of the opening. This conclusion also fits in well with some facts observed at the time of closure of the pieces. Certain of the cells that appear to be more closely stuck to the wall are often left behind, or are retarded in their progress towards the center of the newly forming membrane. Thus the peculiar method of closure of *Tubularia* finds its explanation in the unusually close connection between the perisarc and cœnosarc. I have tried to show elsewhere[1] that this same connection may also be responsible for the characteristic "incomplete structures" of *Tubularia*, whose chief peculiarity is that their organs are full sized so far as they are formed.

TRANSPOSITIONAL OR COMPENSATORY REGENERATION OF THE LARGE CHELÆ IN SOME CRUSTACEA.

Przibram[2] discovered in 1901 in the decapod *Alpheus* that it is possible to cause the small claw (chela) of one side to become the large claw by the simple operation of removing the large claw of the other side. At the next moult the small claw becomes the big one, and the newly regenerated claw becomes the small one. Zeleny[1] found in 1902 that a similar throwing over of the large operculum of the annelid, *Hydroides*, can be brought about by the same sort of operation. Wilson[2] in 1903 made some important additions to Przibram's work, using an American species of *Alpheus*. He suggested that the small claw is merely an arrested stage of development of the big claw, and that when the big claw is removed the check is at the same time taken away that holds back the development of the small claw. At the next moult the small claw becomes the large one, and the new claw the small one.

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[1] "Some Factors in the Regeneration of Tubularia," *Roux's Archiv*, XIV., 1903.

[2] *Roux's Archiv*, XI., 1901.

As yet no one has detected the nature of the correlation that causes the transposition, and this must obviously be the next step in advance. Wilson has suggested that the throwing over is connected with the nervous system, but the experiments on which he bases this suggestion appear to me to be capable also of another interpretation.

During the past summer I undertook some experiments which I hoped would give results bearing on this question, but the outcome has been almost entirely negative. Nevertheless, I shall venture to describe these experiments briefly, because if carried out on more suitable forms they will very probably throw some light on this exceedingly important subject.

Several years ago I found that by cutting the nerve of the leg of the hermit-crab, proximal to the breaking joint, the leg can then be cut off at any level beyond the breaking joint without the remaining part being thrown off at the base. By removing portions of the large leg at different levels, after first cutting the nerve at the base, I hoped to be able to discover whether the amount removed had any effect on the transposition of the large claw to the other side. It was also possible that the simple cutting of the nerve might have some effect, as Wilson's experiment seems to show. The result might also, as Wilson appears to believe, depend in part upon the degree to which the new nerve regenerated before the next moult. In practice, however, it is not possible to cut the nerve without cutting also the blood-vessels, and the injury to the latter may be as important as, or even more so, than that to the nerve.

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[1] *Roux's Archiv*, XIII., 1902.

[2] BIOLOGICAL BULLETIN, IV., 1903.

The experiments were carried out with the hermit-crab and with the fiddler-crab, but were unsuccessful in both cases for different reasons. First, the transposition does not occur under any circumstances in the hermit-crab, as this and other experiments showed; and second, in the fiddler-crabs the muscles, etc., beyond the breaking joint degenerate after the operation. This caused the death of most of the crabs, and those that remained alive had only the outer shell of the leg beyond the breaking joint, and even this fell off in several cases. Since, however, the operation can be carried out in the hermit-crab without the outer part of the leg degenerating, it may be possible, in other forms that have the power of transpositional regeneration (in *Alpheus*, for example), to carry out this experiment successfully.

In both the hermit- and the fiddler-crab I also tried the effect of removing three of the walking legs on the same side of the body as the big claw, leaving the big claw uninjured, in order to see if the absence of the other legs might possibly affect the transposition. This did not succeed, because in the hermit-crabs, as I have said, the big claw does not throw over, and in the fiddlers the experiment had to be brought to an end before any of the crabs had moulted.

All of the individuals of the hermit-crab that I have examined were right-handed, and the shells in which they live have also right-handed spirals. It has been suggested to me that this is an adaptation, in so far that the right-handed hermit crab is placed to better advantage in a right-handed shell. Consequently, if this were true (and I am by no means certain that it is so), it would be disadvantageous for the hermit-crab to have the power of transposition after the loss of the big claw, and in consequence this power has not been acquired, or else, if it existed in the ancestors of the hermit-crabs, it has been lost. That there is really no basis for an argument of this kind is shown by the state of affairs in other decapods; in the lobster, for example. In the American lobster I have seen several cases in which the big claw had been lost and a new one of the same kind was regenerating on the same side. Przibram has also described cases of this sort. This result is all the more interesting since in the lobster the big claw is present in some individuals on one side, and in other individuals on the other. It cannot be claimed in the lobster that one kind of claw represents an undeveloped stage of the other. In the regeneration of the claws, as especially well seen in the lobster, the particular type of claw is present, although not always fully developed, at an early stage, as Przibram has described, and as I have also found. No doubt the advocates of the view that all beneficial processes have been acquired because of the benefit conferred, will find in these cases of transposition of the big claw from one side to the other evidence of the acquirement of a useful process through natural selection, but I do not think that there is any connection of this sort in these cases.[1]

I have intimated above that the injury to the blood-vessels that run to the leg may be closely connected with the changes that take place in the leg, and account for the absence of transposition in those experiments of Wilson's in which the nerve of the small claw was cut (and presumably also the blood-vessel). My work on the fiddler-crab convinced me that cutting the blood-vessels, which seems nearly always to take place when the nerve is cut, brings about important changes in the condition of the leg. If my suggestion prove correct, namely, that the lack of transposition in Wilson's experiment is due to injury to the blood-vessel rather than to cutting the nerve, then it is possible that the whole phenomenon of transposition may be connected with the condition of the blood supply to the leg. After removal of the large claw more blood may be thrown into the vessel going to the small claw, and this may be the cause of the change that takes place.

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[1] In this respect I am in entire agreement with Wilson.

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